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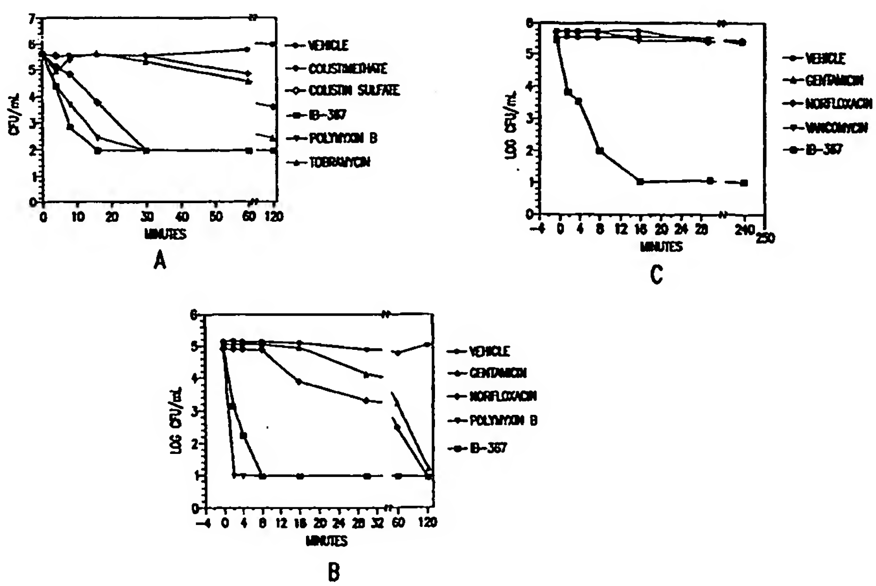

(57) Abstract

The present invention provides compositions and methods for treating or preventing pulmonary infections, particularly pulmonary infections caused by antibiotic–resistant strains of bacteria and/or pulmonary infections in patients at high risk of developing such infections, including patients suffering from chronic obstructive pulmonary disease, bronchiectasis and/or cystic fibrosis.

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COMPOSITIONS AND METHODS FOR THE TREATMENT OR PREVENTION OF PULMONARY INFECTIONS

1. FIELD OF THE INVENTION

The present invention relates to the use of antimicrobial peptides to treat or prevent pulmonary infections. More specifically, the present invention relates to the use of antimicrobial protegrin peptides, and/or congeners or analogs thereof, to treat or prevent pulmonary infections, and in particular pulmonary infections in patients at increased risk for developing lung infections, such as patients suffering from chronic obstructive pulmonary disease, bronchiectasis or cystic fibrosis.

2. BACKGROUND OF THE INVENTION

The airways of the lung are ordinarily sterile, despite the daily inhalation of a variety of organisms. Sterility of the lung is maintained by host defense mechanisms, which include anitmicrobial substances produced by the airway epithelium. Infection can occur when the normal defense mechansims of the lungs become impaired. For instance, in chronic obstructive pulmonary disease (COPD), a syndrome that results from cigarette smoking, excessive mucus production and impaired mucociliary clearance often leads to acute or chronic infection of the airways. Bronchiectasis, a disease in which the airways become dilated and their shapes torturous; is characterized by ineffective clearance of microorganisms from the airways and an increased susceptibility to lung infections. Another disease associated with impaired defense mechanisms and persistent airway infections is cystic fibrosis.

Cystic fibrosis is the most common lethal inherited abnormality in Caucasians (Koch & Hoiby, 1993, Lancet 341: 1065-1069). The disease is caused by mutations in the gene for the cystic fibrosis transmembrane conductance regulator

(CFTR). The CFTR gene encodes a chloride channel; defects in the encoded protein result in an increased concentration of sodium chloride in the airway surface fluid. It is believed that this increased sodium chloride concentration is one of the causes of the thickened, retained mucus secretions that characterize cystic fibrosis.

A variety of therapeutic approaches for reducing these retained mucus secretions exist. For example, the use of aerosolized amiloride to facilitate removal of retained mucus secretions is described in U.S. Patent No. 4,501,729. The administration of adenosine triphosphate or uridine triphosphate to induce hydrated secretions is described in Stock, 1992, Endeavors 10:10-11. The use of lantibiotics such as duramycin to facilitate clearance of retained pulmonary secretions is described in U.S. Patent Nos. 5,683,675 and 5,651,957. Lastly, it has recently been discovered that administration of DNases reduces the viscosity and retention of the secretions (Shak *et al.*, 1996, Thorax 51:119-125).

However, while the retained mucus secretions are a major cause of morbidity in patients suffering from cystic fibrosis, the most common cause of mortality is bacterial infection in the airways. For example, patients with cystic fibrosis typically suffer from recurrent and/or persistent lung infections caused by any of several species of bacteria, including *P. aeruginosa, S. aureus and H. influenza*. In many instances, the infections are caused by bacteria which have developed resistance to common antibiotics, such as methicillin-resistant *S. aureus* (MRSA) and tobramycin-resistant *P. aeruginosa* (TRPA).

Given the prevalence of lung infection in patients with conditions such as COPD, bronchiectasis and/or cystic fibrosis, treatment or prophylaxis of these pulmonary infections is an important aspect of treatment regimens for these conditions. In the case of cystic fibrosis, treatment of the infections oftentimes also improves lung

function. For example, it has been shown that administration of inhaled tobramycin reduces sputum bacterial density and improves pulmonary function in patients with cystic fibrosis (Fiel, 1995, Chest. 107:615-645).

Treatment of persistent and/or recurrent lung infections in patients with COPD, bronchiectasis and/or cystic fibrosis presents unique challenges. In the case of cystic fibrosis, the high incidence of infection, like the retained mucus secretions, is also thought to be related to the increased salt concentration of the airway surface fluid. A recent study demonstrated that airway epithelial cells from both normal subjects and subjects with cystic fibrosis produce an as yet uncharacterized endogenous antimicrobial substance that is able to kill *P. aeruginosa* under conditions of low salt concentration (Smith *et al.*, 1996, Cell 85:228-236). However, this endogenous antimicrobial substance is unable to kill *P. aeruginosa* cultured on the surface of airway epithelial cells from patients with cystic fibrosis, owing to the high salt concentration of the surface fluid and the salt sensitivity of the antimicrobial substance (Smith *et al.*, *supra*). Thus, agents used to combat pulmonary infections in cystic fibrosis patients must be active under conditions of high salinity.

Moreover, the increasing prevalence of resistance to antibiotics has proven problematic for the treatment of lung infections generally, and in particular in patients with COPD, bronchiectasis and/or cystic fibrosis. Not only are infections often caused by antibiotic-resistant strains of bacteria (*e.g.*, MRSA and TRPA in patients with cystic fibrosis and penicillin-resistant *S. pneumoniae* (PRSP) in patients with COPD or bronchiectasis), the recurrent and persistent nature of the infections observed in these patients tends to engender antibiotic resistance (for a review, *see*, Rosenfeld *et al.*, 1998, Infectious Diseases in

Clinical Practice 7:66-79). Thus, agents used to combat pulmonary infections in patients with these disorders should have broad spectrum activity against even antibiotic-resistant strains of bacteria, and also exhibit a low frequency of resistance induction under prolonged and/or frequent use.

As the above discussion attests, there remains a need in the art for broad spectrum antimicrobial agents useful for treating pulmonary infections, particularly those caused by *P. aeruginosa*, *S. aureus*, *H. influenza*, *S. pneumoniae* and/or antibiotic resistant pathogens such as MRSA, TRPA and/or PRSP. There also remains a need in the art for broad-spectrum antimicrobial agents that are effective against the pathogens associated with cystic fibrosis under the conditions of high salinity observed in the lungs of cystic fibrosis patients. Additionally, there remains a need in the art for broad spectrum antimicrobial agents which engender little resistance with frequent and/or prolonged use. Accordingly, these are objects of the present invention.

3. SUMMARY OF THE INVENTION

These and other objects are furnished by the present invention, which in one aspect provides a method for treating or preventing pulmonary infections in animals, including humans. The method generally comprises administering to an animal subject an antimicrobial protegrin peptide, or a pharmaceutically acceptable salt thereof, in an amount effective to combat the bacterial infection, particularly infections caused by *P. aeruginosa*, *S. aureus*, *H. influenza*, and *S. pneumoniae* and/or antibiotic resistant strains of bacteria such as MRSA TRPA and/or PRSP. The antimicrobial protegrin peptide is preferably administered locally to the lungs of the subject in aerosolized form *via* inhalation. The protegrin peptide may be administered singly, in combination with one or more

other protegrin peptides or in combination with other agents commonly used to treat or prevent infections, such as conventional antibiotics. The method is particularly useful to treat or prevent infections in patients having an increased risk of suffering persistent and/or recurrent lung infections, such as patients suffering from COPD, bronchiectasis and/or cystic fibrosis. The protegrin peptide may be administered prophylactically to such patients prior to the onset of infection, or therapeutically after the emergence of infection.

In another aspect, the present invention provides methods of treating cystic fibrosis. The method generally involves administering to the lungs of a subject having cystic fibrosis an antimicrobial protegrin peptide, or a pharmaceutically effective salt thereof, in an amount effective to treat cystic fibrosis. The antimicrobial protegrin peptide is preferably administered locally to the lungs of the patient in aerosolized form *via* inhalation. The antimicrobial protegrin peptide may be administered singly, in combination with one or more other protegrin peptides, in combination with other agents commonly used to treat cystic fibrosis, such as PULMOZYME® (Genentech), or in combination with other agents commonly used to treat the chronic and/or recurrent infections associated with cystic fibrosis, such as tobramycin and/or colistin.

In a final aspect, the present invention provides a pharmaceutical composition comprising an antimicrobial protegrin peptide and a pharmaceutically acceptable carrier, excipient and/or diluent. The composition is useful for aerosolized application of the protegrin peptide to the lungs of a subject in conjunction with the methods of the invention.

4. BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1C are graphs illustrating the time-dependent bactericidal activity of a variety of antimicrobial agents

against *P. aeruginosa* or *S. aureus*. Cultures were incubated with antimicrobial agent and the number of viable organisms, based on an evaluation of colony forming units (CFU), was determined at specified times. In FIG. 1A, ● represents vehicle (control); ♦ represents 16 μg/ml colistimethate; ◊ represents 1 μg/ml colistin sulfate; ■ represents 4 μg/ml IB-367 (RGGLCYCRGRFCVCVGR-NH_2 SEQ ID NO:6; ▾ represents 1 μg/ml polymyxin B; and ▴ represents 0.5 μg/ml tobramycin. In FIG. 1B, ● represents vehicle (control); ▴ represents 1 μg/ml gentamicin; ♦ represents 1 μg/ml norfloxacin; ▾ represents 1 μg/ml polymyxin B; and ■ represents 4 μg/ml peptide IB-367 (SEQ ID NO:6). In FIG. 1C, ● represents vehicle (control); ▴ represents 1 μg/ml gentamicin; ♦ represents 1 μg/ml norfloxacin; ▾ represents 1 μg/ml vancomycin; and ■ represents 4 μg/ml peptide IB-367 (SEQ ID NO:6). All concentrations are approximately two times the minimum inhibitory concentration (MIC) of the respective agent against the particular bacterial species.

FIG. 2 is a graph illustrating the bactericidal activity of a variety of antimicrobial agents against *P. aeruginosa* in bronchoalveolar lavage fluid (BALF) from cystic fibrosis patients. *P. aeruginosa* was added to previously frozen BALF to yield a density of approximately 1×10^8 CFU/ml, antimicrobial agents were added at concentrations of 100 μg/ml and the cultures were incubated at 37°C. The number of viable organisms, based on an evaluation of CFU, was determined at specified times. ● represents vehicle; ♦ represents colistimethate; ◊ represents colistin sulfate; ▴ represents tobramycin; and ■ represents IB-367 (SEQ ID NO:6).

FIG. 3A is a graph illustrating the bactericidal activity of various concentrations of protegrin peptide IB-367 (SEQ ID NO:6) against endogenous flora in sputum pooled from patients with cystic fibrosis. Peptide IB-367 (SEQ ID NO:6) was mixed 1:1 to the pooled sputum to give the indicated final concentrations. The number of viable

organisms, based on an evaluation of CFU, was determined at specified times. ● represents vehicle; ♦ represents 250 μg/ml IB-367 (SEQ ID NO:6); ▾ represents 1000 μg/ml IB-367 (SEQ ID NO:6); and ■ represents 4000 μg/ml IB-367 (SEQ ID NO:6).

FIG. 3B is a graph illustrating the bactericidal activity of a variety of antimicrobial agents against endogenous flora in sputum pooled from patients with cystic fibrosis. Sputum was mixed 1:1 with a solution of mannitol:sucrose (3%:3%) and mixed vigorously. Antimicrobial agents were mixed 1:1 with the sputum mixture to give a final concentration of 1000 μg/ml, mixed vigorously and incubated at 37°C. The number of viable organisms, based on an evaluation of CFU, was determined at specified times. ● represents vehicle; ♦ represents colistimethate; ▾ represents polymyxin B; ■ represents IB-367 (SEQ ID NO:6); and ▴ represents tobramycin.

FIG. 3C is a graph illustrating that the kill kinetics of IB-367 (SEQ ID NO:6) in sputum pooled from patients with cystic fibrosis improved upon readdition of drug at 120 min. (line). ● represents vehicle; ■ represents 1 mg/ml IB-367; ♦ represents 1 mg/ml colistimethate; and ▴ represents 1 mg/ml tobramycin.

FIGS. 4A and 4B provide graphs illustrating the resistance of *P. aeruginosa* and *S. aureus* induced by various antimicrobial agents. In FIG. 4A, ♦ represents norfloxacin; ▾ represents polymyxin B; and ■ represents protegrin peptide IB-367 (SEQ ID NO:6). In FIG. 4B, ♦ represents norfloxacin; ▾ represents vancomycin; and ■ represents protegrin peptide IB-367 (SEQ ID NO:6).

FIG. 5 provides a graph illustrating the antimicrobial activity of peptides IB-367 (SEQ ID NO:6) and IB-734 (ZGGZLCYCZZZFCVCVGZ-NH_2, where Z is Dbu; SEQ ID NO:9) against *P. aeruginosa* on monolayer cultures of Calu-3 epithelial cells.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1 ABBREVIATIONS

The amino acid notations used herein for the twenty genetically encoded amino acids are conventional and are as follows:

Amino Acid	One-Letter Symbol	Common Abbreviation
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamine	Q	Gln
Glutamic acid	E	Glu
Glycine	G	Gly
Histidine	H	His
Isoleucine	I	Ile
Leucine	L	Leu
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val

The abbreviations used for the D-enantiomers of the genetically encoded amino acids are lower-case equivalents of the one-letter symbols. For example, "R" designates L-arginine and "r" designates D-arginine. The three letter abbreviations are not intended to define a particular

stereochemistry. Thus, when the three-letter abbreviations are used the amino acid may be either an L-amino acid or a D-amino acid.

5.2 DEFINITIONS

As used herein, the following terms shall have the following meanings:

"Protegrin Peptide:" refers to one of the five naturally-occurring antimicrobial peptides originally isolated from porcine leukocytes (designated PG-1 through PG-5) described in WO 96/37508. The five naturally occurring protegrin peptides are characterized by an amphipathic β-sheet structure and two disulfide bridges--one between residues C_6 and C_{15} and another between residues C_8 and C_{13}. The naturally occurring protegrin peptides are amidated at the C-terminus. However, peptides which differ in the number and pattern of disulfide bridges, which contain more or fewer amino acid residues and/or which vary in other respects from the five naturally-occurring protegrin peptides also possess significant antimicrobial activity. Thus, also included within the definition of "protegrin peptides" are active congeners and analogs of the five naturally-occurring protegrin peptides, as defined herein.

"Congener:" refers to an antimicrobial peptide which has an amino acid sequence that is different from a naturally-occurring protegrin peptide, but which retains a sufficient number of the characteristics of the naturally-occurring protegrin peptides so as to be recognized as belonging to the same class. While one of the characteristics of the naturally-occurring protegrin peptides is the presence of two disulfide bridges, congeners of protegrin peptides may contain two, one or zero disulfide bridges and may have a disulfide bridging pattern that differs from that of the naturally-occurring

peptides. Protegrin peptide congeners may also contain more or fewer amino acid residues than the naturally-occurring protegrin peptides, and may be cyclized by way of backbone atoms. Exemplary protegrin peptide congeners are described, for example, in U.S. Patent No. 5,464,823; U.S. Patent No. 5,693,486; U.S. Patent No. 5,708,145; application Serial No. 08/984,294, filed December 3, 1997; WO 95/03325; WO 96/37508; WO 97/18826; and WO 97/18827. Exemplary cyclized protegrin peptide congeners are described in WO 98/03192 and U.S. application Serial No. 08/685,589, filed July 24, 1996.

"<u>Analog</u>:" refers to a compound having the amino acid sequence of a naturally-occurring protegrin peptide or protegrin peptide congener, but in which at least one of the backbone amide interlinkages [-C(O)NH-] is replace with another interlinkage, such as a substituted amide [-C(O)NR-, where R is (C_1-C_8) alkyl], an isostere of an amide or a peptidomimetic moiety. Also included within the definition of "analog" are forms of the various protegrin peptides described herein which are modified at their N- and/or C-terminus. Specifically included within the N- and/or C-terminal modified forms are protegrin peptides in which the N-terminus is of the formula R-C(O)-NH- and/or the C-terminus is of the formula -C(O)OH, -C(O)OR, -C(O)NH_2, -C(O)NHR or -C(O)NRR, where each R is independently (C_1-C_8) alkyl preferably (C_1-C_3) alkyl.

5.3 <u>THE INVENTION</u>

The present invention provides compositions and methods for the treatment or prevention of pulmonary infections in animals, including humans. While the compositions and methods described herein can be used to treat infections caused by virtually any pathogen against which the active compounds exert antimicrobial activity in virtually any subject, of particular interest is the treatment or prevention of infections in patients at high

risk of suffering recurrent and/or persistent infections, such as patients suffering from COPD, bronchiectasis and/or cystic fibrosis.

Moreover, as the compounds described herein do not engender resistance even during repeated and/or prolonged use, an important aspect of the present invention is the ability to treat or prevent pulmonary infections caused by strains of bacteria that are resistant to, or readily develop resistance to, conventional antibiotics. Antibiotic-resistant strains of bacteria of particular interest against which the compositions are active include, *e.g.*, methicillin-resistant *S. aureus* (MRSA) tobramycin-resistant *P. aeruginosa* (TRPA) and penicillin-resistant *S. pneumoniae* (PRSP). Conventional antibiotics commonly used to treat pulmonary infections which typically engender resistance when used improperly or for prolonged periods include, for example, aminoglycosides (*e.g.*, tobramycin), β-lactams (*e.g.*, cephalosporins, carbenicillin, etc.) and quinolones (*e.g.*, ciprofloxacin).

When the compounds are used to treat or prevent pulmonary infections in patients with cystic fibrosis, consequential reductions in the symptoms associated with cystic fibrosis may also be achieved. For example, administration of the compositions will often times result in improved lung function, reduction in fever, etc. Thus, the invention also provides compositions and methods for the treatment of cystic fibrosis.

5.3.1 THE COMPOUNDS

Active compounds which can be used to treat or prevent pulmonary infections and/or cystic fibrosis according to the methods of the invention are protegrin peptides, which include the active analogs and congeners thereof as defined herein. As discussed in the Background section, persistent and recurrent pulmonary infections caused by pathogens such

as *S. pneumoniae* in patients with COPD and/or bronchiectasis and *P. aeruginosa*, *S. aureus*, and *H. influenza* in patients with cyctic fibrosis, are a significant cause of morbidity and mortality in these patients. As also discussed in the Background section, the high salinity of the airway surface fluid in patients with cystic fibrosis is thought to be a major contributor to the persistent and recurrent pulmonary infections observed in patients with cystic fibrosis, as many endogenous antimicrobial agents are not active under conditions of high salt concentration.

The protegrin peptides are a recognized class of naturally-occurring antimicrobial peptides that exhibit broad spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, yeast, fungi and certain viruses (for a review of the properties of protegrin peptides, (*see*, WO 96/37508 and the references cited therein). To date, five different naturally-occurring protegrin peptides have been isolated from porcine leukocytes. These protegrin peptides are designated PG-1 through PG-5 and have the following amino acid sequences:

(PG-1)	RGGRLCYCRRRFCVCVGR-NH_2	(SEQ ID NO:1)
(PG-2)	RGGRLCYCRRRFCICV-NH_2	(SEQ ID NO:2)
(PG-3)	RGGGLCYCRRRFCVCVGR-NH_2	(SEQ ID NO:3)
(PG-4)	RGGRLCYCRGWICFCVGR-NH_2	(SEQ ID NO:4)
(PG-5)	RGGRLCYCRPRFCVCVGR-NH_2	(SEQ ID NO:5)

Naturally-occurring protegrins PG-1 through PG-5 are amidated at the C-terminus and have two disulfide linkages-- one between residues C_6 and C_{15} and another between residues C_8 and C_{13}.

Recently, a number of congeners and analogs of the naturally-occurring protegrin peptides, as well as cyclized forms of protegrin peptides, have been designed (*See* U.S. Patent No. 5,464,823; U.S. Patent No. 5,693,486; U.S.

Patent No. 5,708,145; application Serial No. 08/984,294, filed December 3, 1997; WO 95/03325; WO 96/37508; WO 97/18826; WO 97/18827; WO 98/03192 and U.S. application Serial No. 08/685,589, filed July 24, 1996). These protegrin peptides differ from the naturally-occurring protegrins in a variety of respects. For example, many of these protegrins are not amidated at the C-terminus, or contain other C-terminal or N-terminal modifications such as C-terminal ester groups and/or N-terminal acetyl groups. In addition many of these protegrin peptides contain more or fewer amino acid residues than the naturally-occurring protegrins, or contain different numbers or patterns of disulfide bridges from the naturally-occurring protegrins. These protegrins, like the naturally- occurring protegrins PG-1 through PG-5, also exhibit broad spectrum antimicrobial activity.

It has been discovered that protegrin peptides are capable of exerting their broad spectrum antimicrobial activity against pathogens that cause pulmonary infections under the conditions of high salinity observed in the airway fluid of patients with cystic fibrosis, making them ideal agents to treat or prevent lung infections in patients suffering from cystic fibrosis. Additionally, unlike traditional antibiotic agents, protegrin peptides exhibit a low frequency of resistance induction, making them ideally suited for long-term prophylactic use, for therapeutic use in treating persistent and/or recurrent infections and/or for treating or preventing infections caused by antibiotic-sensitive or antibiotic-resistant strains of bacteria.

Protegrin peptides which are useful for treating or preventing pulmonary infections according to the invention include the five naturally-occurring protegrins, as well as the active analogs and congeners thereof. Suitable protegrin peptides, analogs and congeners are described, for example, in U.S. Patent No. 5,464,823, U.S. Patent No. 5,693,486, U.S. Patent No. 5,708,145, application Serial

No. 0/984,294, filed December 3, 1997, WO 95/03325, WO 96/37508, WO 97/18826 and WO 97/18827. Cyclized protegrin peptide congeners, which are particularly suited for use against gram-negative pathogens, are described in application Serial No. 08/685,589 and WO 98/03192. Any of these protegrins can be used in accordance with the methods of the invention. Thus, protegrin peptides suitable for use with the methods of the invention are either known to those of skill in the art or will be easily identified by way of tests commonly employed in the art, such as, for example, the tests provided in the examples.

Generally, protegrin peptides useful in the methods of the invention will exhibit a minimum inhibitory concentration (MIC) against the pathogen to be combatted of less than about 64 μg/ml, preferably less than about 32 μg/ml, more preferably less than about 16 μg/ml, or even lower, as measured using the modified assays described in WO 97/18826 and/or Steinberg et al., 1997, Antimicrob. Agents Chemother. 41:1738-1742, or the assays described in the examples. In particularly preferred embodiments, the protegrin peptide will have an MIC against the target pathogen of equal to or less than about 8 μg/ml.

Alternatively, or in addition, useful protegrin peptides will generally induce at least a one log reduction in respiratory colony forming units (CFU) of the target pathogen within about 60 minutes of being delivered locally to the lungs *via* a solution or powder formulation containing peptide at a concentration in the range of about 0.1% (w/v) to 10% (w/v), preferably in the range of about 0.1% (w/v) to 1% (w/v).

Of course, when used to treat or prevent pulmonary infections in patients with cystic fibrosis, the protegrin peptides should exert their antimicrobial activity under conditions of high salinity, such as, for example, in 100 mM to 200 mM sodium chloride. Determination of antimicrobial activity under appropriate conditions is well within the capabilities of these having skill in the art.

In a preferred embodiment of the invention, the protegrins are 12-18 amino acid residue peptides having the structural formula (I):

(I) X_1-X_2-X_3-X_4-X_5-C_6-X_7-C_8-X_9-X_{10}-X_{11}-X_{12}-C_{13}-X_{14}-C_{15}-X_{16}-X_{17}-X_{18}

wherein:

X_1 is a basic amino acid (preferably Arg or Dbu) or absent;

X_2 is a hydrophobic amino acid or absent;

X_3 is a hydrophobic amino acid or absent;

X_4 is a basic amino acid (preferably Arg or Dbu) or absent;

X_5 is an aliphatic amino acid (preferably Leu or Cha);

each of C_6, C_8, C_{13} and C_{15} is independently selected from the group consisting of a cysteine-like amino acid (preferably Cys) and a polar amino acid (preferably a hydroxyl-containing amino acid such as Ser or Thr);

X_7 is an aromatic amino acid (preferably Tyr);

X_9 is a basic amino acid (preferably Arg or Dbu);

X_{10} is a basic amino acid (preferably Arg or Dbu) or a helix-breaking amino acid (preferably Gly or Pro);

X_{11} is a basic amino acid (preferably Arg or Dbu);

X_{12} is an aromatic amino acid (preferably Phe);

X_{14} is an aliphatic amino acid (preferably Val);

X_{16} is an aliphatic amino acid (preferably Val);

X_{17} is an aliphatic amino acid (preferably Gly) or absent; and

X_{18} is a basic amino acid (preferably Arg or Dbu) or absent.

In structure (I), each X_n and C_n designate an amino acid residue belonging to two main classes: hydrophobic and hydrophilic. These two main classes can be further classified into sub-classes, with the hydrophilic class including acidic, basic and polar sub-classes and the hydrophobic class including aromatic, apolar and aliphatic

sub-classes. Another category that defines residues X_n is the class of helix-breaking residues. Each C_n in structure (I) further defines those amino acid residues which may optionally participate in disulfide-bridges, designated "cysteine-like" amino acids. The various classifications of the amino acids composing the peptides of structure (I) are defined below.

"Hydrophilic Amino Acid:" refers to an amino acid exhibiting a hydrophobicity of less than zero according to the normalized consensus hydrophobicity scale of Eisenberg et al., 1984, J. Mol. Biol. 179:125-142. Genetically encoded hydrophilic amino acids include Thr (T), Ser (S), His (H), Glu (E), Asn (N), Gln (Q), Asp (D), Lys (K) and Arg (R).

"Acidic Amino Acid:" refers to a hydrophilic amino acid having a side chain pK value of less than 7. Acidic amino acids typically have negatively charged side chains at physiological pH due to loss of a hydrogen ion. Genetically encoded acidic amino acids include Glu (E) and Asp (D).

"Basic Amino Acid:" refers to a hydrophilic amino acid having a side chain pK value of greater than 7. Basic amino acids typically have positively charged side chains at physiological pH due to association with hydronium ion. Genetically encoded basic amino acids include His (H), Arg (R) and Lys (K).

"Polar Amino Acid:" refers to a hydrophilic amino acid having a side chain that is uncharged at physiological pH, but which has at least one bond in which the pair of electrons shared in common by two atoms is held more closely by one of the atoms. Genetically encoded polar amino acids include Asn (N), Gln (Q) Ser (S) and Thr (T).

"Hydrophobic Amino Acid:" refers to an amino acid exhibiting a hydrophobicity of greater than zero according to the normalized consensus hydrophobicity scale of Eisenberg, 1984, J. Mol. Biol. 179:125-142. Genetically encoded hydrophobic amino acids include Pro (P), Ile (I), Phe (F), Val (V), Leu (L), Trp (W), Met (M), Ala (A), Gly (G) and Tyr (Y).

"Aromatic Amino Acid:" refers to a hydrophobic amino acid having a side chain with at least one aromatic or heteroaromatic ring. The aromatic or heteroaromatic ring may contain one or more substituents, such as -R, -OH, -SH, -CN, -F, -Cl, -Br, -I, $-NO_2$, -NO, $-NH_2$, -NHR, -NRR, -C(O)R, -C(O)OH, -C(O)OR, $-C(O)NH_2$, -C(O)NHR, -C(O)NRR, and the like, where each R is independently (C_1-C_8) alkyl. Genetically encoded aromatic amino acids include Phe (F), Tyr (Y) and Trp (W).

"Nonpolar Amino Acid:" refers to a hydrophobic amino acid having a side chain that is uncharged at physiological pH and which has bonds in which the pairs of electrons shared in common by two atoms are generally held equally by each of the two atoms (*i.e.*, the side chain is not polar). Genetically encoded apolar amino acids include Leu (L), Val (V), Ile (I), Met (M), Gly (G) and Ala (A).

"Aliphatic Amino Acid:" refers to a hydrophobic amino acid having an aliphatic hydrocarbon side chain. Genetically encoded aliphatic amino acids include Ala (A), Val (V), Leu (L) and Ile (I). While Gly (G) does not have a side chain, it is also included in the aliphatic class of amino acids.

"Cysteine-like Amino Acid:" The amino acid residue Cys (C) is unusual in that it can form disulfide bridges with other Cys (C) residues or other sulfanyl-containing amino acids. Due to the ability of Cys (C) residues (and

other amino acids with -SH side chains) to exist in a peptide in either a reduced free -SH or oxidized disulfide-bridged form, these residues are not included within the above-delineated classes, but rather form their own class of amino acids called "cysteine-like" amino acids. Any amino acid residue having a side chain capable of participating in a disulfide bridge is included within the class of cysteine-like amino acids.

As will be appreciated by those of skill in the art, the above-defined categories are not mutually exclusive. Thus, amino acids having side chains exhibiting two or more physico-chemical properties can be included in multiple categories. For example, amino acid side chains having aromatic moieties that are further substituted with polar substituents, such as Tyr (Y), may exhibit both aromatic hydrophobic properties and polar hydrophilic properties, and can therefore be included in both the aromatic and polar categories. The appropriate categorization of any amino acid will be apparent to those of skill in the art, especially in light of the detailed disclosure provided herein.

Certain amino acid residues, called "helix breaking" amino acids, have a propensity to disrupt the structure of α-helices when contained at internal positions within a helix. Amino acid residues exhibiting such helix-breaking properties are well-known in the art (*see, e.g.*, Chou and Fasman, Ann. Rev. Biochem. 47:251-276), and include Pro (P), Gly (G) and potentially all D-amino acids (when contained in an L-peptide; conversely, L-amino acids disrupt helical structure when contained in a D-peptide). It has been found that while the protegrin peptides are β-sheet rather than α-helical in nature, these helix-breaking residues impart important structural characteristics when contained at residue position X_{10}, which is within a β-turn region of the peptide.

While the above-defined categories have been exemplified in terms of the genetically encoded amino

acids, the amino acid residues comprising the various classes need not be, and in certain embodiments preferably are not, restricted to the genetically encoded amino acids. Indeed, many of the preferred peptides of structure (I) contain genetically non-encoded amino acids. Certain commonly encountered non-encoded amino acids which can be used in structure (I) include, but are not limited to, β-alanine (β-Ala) and other omega-amino acids such as 3-aminopropionic acid (Apr), 2,3-diaminopropionic acid (Dpr), 4-aminobutyric acid (Abu) and so forth; α-aminoisobutyric acid (Aib); ε-aminohexanoic acid (Aha); δ-aminovaleric acid (Ava); N-methylglycine or sarcosine (MeGly); ornithine (Orn); citrulline (Cit); t-butylalanine (t-BuA); t-butylglycine (t-BuG); N-methylisoleucine (MeIle); phenylglycine (Phg); cyclohexylalanine (Cha); norleucine (Nle); naphthylalanine (Nal); 4-chlorophenylalanine (Phe-4-Cl); 2-fluorophenylalanine (Phe-2-F); 3-fluorophenylalanine (Phe-3-F); 4-fluorophenylalanine (Phe-4-F); penicillamine (Pen); 1,2,3,4tetrahydroisoquinoline-3-carboxylic acid (Tic); β-2-thienylalanine (Thi); methionine sulfoxide (MSO); homoarginine (hArg); N-acetyl lysine (AcLys); 2,4-diaminobutyric acid (Dbu); 2,3-diaminobutyric acid (Dab); *p*-aminophenylalanine (Phe-*p*NH_2); N-methyl valine (MeVal); homocysteine (hCys), homophenylalanine (hPhe), homoserine (hSer); hydroxyproline (Hyp), and homoproline (hPro).

The classifications of the genetically encoded and common non-encoded amino acids according to the categories defined above are summarized in TABLE 1, below. It is to be understood that TABLE 1 is for illustrative purposes only and does not purport to be an exhaustive list of amino acid residues that can compose the protegrin peptides of structure (I). Other amino acid residues not specifically mentioned can be readily categorized based on their observed physical and chemical properties in light of the definitions provided herein.

TABLE 1
CLASSIFICATIONS OF COMMONLY ENCOUNTERED AMINO ACIDS

Classification	Genetically Encoded	Non-Genetically Encoded
Hydrophobic		
Aromatic	F, Y, W	Phg, Nal, Thi, Tic, Phe-4-Cl, Phe-2-F, Phe-3-F, Phe-4-F, hPhe
Apolar	L, V, I, M, G, A, P	t-BuA, t-BuG, MeIle, Nle, MeVal, Cha, MeGly, Aib
Aliphatic	G, A, V, L, I	b-Ala, Aib, Aha, MeGly, t-BuA, t-BuG, MeIle, Cha, Nle, MeVal
Hydrophilic		
Acidic	D, E	
Basic	H, K, R	Dpr, Orn, hArg, Phe-pNH_2, Dbu, Dab
Polar	Q, N, S, T	Cit, AcLys, MSO, hSer
Helix-Breaking	P, G	D-Pro and other D-amino acids (in L-peptides)
Cysteine-like	C	Pen, hCys

In the protegrin peptides of structure (I), the symbol "—" between amino acid residues X_n and/or C_n generally designates a backbone constitutive linking function. Thus, the symbol "-" usually represents a peptide bond or amide linkage (-C(O)NH-). It is to be understood, however, that the present invention contemplates protegrin peptide analogs wherein one or more amide linkages is optionally replaced with a linkage other than amide, preferably a substituted amide or an isostere of amide. Thus, while the various X_n and C_n residues within structure (I) are generally described in terms of amino acids, and preferred embodiments of the invention are exemplified by way of peptides, one having skill in the art will recognize that in embodiments having non-amide linkages, the term "amino acid" or "residue" as used herein refers to other

bifunctional moieties bearing groups similar in structure to the side chains of the amino acids.

Substituted amides generally include, but are not limited to, groups of the formula -C(O)NR-, where R is (C_1-C_9) alkyl. Isosteres of amide generally include, but are not limited to, -CH_2NH-, -CH_2S-, -CH_2CH_2-, -CH=CH- (cis and trans), -C(O)CH_2-, -CH(OH)CH_2- and -CH_2SO-. Compounds having such non-amide linkages and methods for preparing such compounds are well-known in the art (*see, e.g.*, Spatola, March 1983, Vega Data Vol. 1, Issue 3; Spatola, 1983, "Peptide Backbone Modifications" *In*: Chemistry and Biochemistry of Amino Acids Peptides and Proteins, Weinstein, ed., Marcel Dekker, New York, p. 267 (general review); Morley, 1980, Trends Pharm. Sci. 1:463-468; Hudson et al., 1979, Int. J. Prot. Res. 14:177-185 (-CH_2NH-, -CH_2CH_2-); Spatola et al., 1986, Life Sci. 38:1243-1249 (-CH_2-S); Hann, 1982, J. Chem. Soc. Perkin Trans. I. 1:307-314 (-CH=CH-, cis and trans); Almquist *et al.*, 1980, J. Med. Chem. 23:1392-1398 (-COCH_2-); Jennings-White *et al.*, Tetrahedron. Lett. 23:2533 (-COCH_2-); European Patent Application EP 45665 (1982) CA 97:39405 (-CH(OH)CH_2-); Holladay *et al.*, 1983, Tetrahedron Lett. 24:4401-4404 (-C(OH)CH_2-); and Hruby, 1982, Life Sci. 31:189-199 (-CH_2-S-).

Additionally, one or more amide linkages can be replaced with peptidomimetic or amide mimetic moieties which do not significantly interfere with the structure or activity of the peptides. Suitable amide mimetic moieties are described, for example, in Olson et al., 1993, J. Med. Chem. 36:3039-3049.

The naturally-occurring protegrin peptides contain two disulfide bridges: one between residues C_6-C_{15} and another between residues C_8-C_{13} (when aligned to the sequence of protegrin PG-1). However, forms of protegrin peptides having other permutations of disulfide bridges (*e.g.*, C_6-C_8 and C_{13}-C_{15}; or C_6-C_{13} and C_8-C_{15}), forms having a single

disulfide bridge (*e.g.*, C_6-C_8; C_6-C_{13}; C_6-C_{15}; C_8-C_{13}; or C_8-C_{15}), and forms having zero disulfide bridges are active and are within the scope of the invention. When linear forms (*i.e.*, forms containing zero disulfide bridges) are desired, the peptide can be "SH-stabilized" by reacting the sulfanyl groups of the cysteine-like residues with alkylating agents using well-known methods. Such "SH-stabilized" forms are incapable of forming intra or intermolecular disulfide bridges. Alternatively, the cysteine-like residues can be replaced with amino acids that do not contain sulfanyl groups and which are therefore incapable of forming disulfide linkages. SH-stabilized forms of protegrin peptides, especially protegrins according to structure (I), which are particularly effective against Gram negative bacteria are those wherein one or more, and preferably all four, cysteine-like residues are replaced with a hydroxyl-containing amino acid such as threonine or serine, preferably threonine.

Naturally-occurring protegrin peptides are C-terminal amidated (*i.e.*, the C-terminus has the structure $-C(O)NH_2$). However, also useful in the present methods are those analogs of the protegrin peptides, particularly those according to structure (I), wherein the C-terminus has the formula -C(O)R, -C(O)OR or -C(O)NRR and/or wherein the N-terminus has the formula R-C(O)-NH-, where each R is independently selected from the group consisting of -H and (C_1-C_8) alkyl.

One particularly useful class of protegrin peptides for treating or preventing pulmonary infections, particularly those caused by *P. aeruginosa, S. aureus, H. influenza, S. pneumoniae, MRSA, TRPA* and/or *PRSP* or pulmonary infections in patients having COPD, bronchiectasis and/or cystic fibrosis, are protegrin peptides according to structure (I) in which X_3 is an aromatic amino acid (preferably Trp) and X_2 is an aliphatic amino acid (preferably Gly) or absent.

Another particularly useful class of protegrin peptides are peptides according to structure (I) in which X_2 is an aliphatic amino acid (preferably Gly) and X_3 is an aliphatic amino acid (preferably Gly).

Still another particularly useful class of protegrin peptides are peptides according to structure (I) in which X_1, X_2, and X_4, X_{17} and X_{18} are absent.

Still another particularly useful class of protegrins peptides are peptides according to structure (I) in which X_1, X_2, X_3, X_4, X_{17} and X_{18} are each absent. Particularly preferred protegrins according to this aspect of the invention are those in which X_5 is Cha.

Still another particularly useful class of protegrin peptides are peptides according to structure (I) in which X_4 is absent and X_{10} is a helix-breaking amino acid (preferably Gly).

Still another particularly useful class of protegrin peptides are peptides according to structure (I) in which X_1, X_2 and X_4 are absent; X_3 is aromatic (preferably Trp); and/or X_{17} and X_{18} are absent.

Yet another particularly useful class of protegrins peptides are peptides according to structure (I) in which X_1, X_4, X_9, X_{10}, X_{11} and X_{18} are each Arg or Dbu.

Yet another particularly useful class of protegrin peptides are peptides according to structure (I) which are selected from the group consisting of:

(PG-1)	RGGRLCYCRRRFCVCVGR-NH_2	(SEQ ID NO:1);
(IB-367)	RGG~LCYCRGRFCVCVGR-NH_2	(SEQ ID NO:6);
(IB-315)	XCYCRRRFCVCV-NH_2	(SEQ ID NO:7);
(IB-482)	W~LCYCZZZFCVCV-NH_2	(SEQ ID NO:8);
(IB-734)	ZGGZLCYCZZZFCVCVGZ-NH_2	(SEQ ID NO:9);

and the N-terminal acylated and/or C-terminal acid or esterified forms thereof, wherein X is Cha, Z is Dbu and "~" designates a missing residue.

Preferred amongst the above-delineated peptides SEQ ID NOS: 1, 6, 7, 8 and 9 are those forms which have two disulfide bridges: one between C_6 and C_{15} and another between residues C_8 and C_{13} (when aligned to the sequence of protegrin PG-1).

While not intending to be bound by any particular theory of operation, it is believed that the protegrin peptides exert their antimicrobial activity as multimers, and that the amphipathic nature of the β-sheet is one factor that contributes to the ability of the protegrins to form active multimers. As will be recognized by those having skill in the art, while preferably the peptides are composed wholly of L-amino acids, one or more L-amino acids can be replaced with the corresponding D-isomers without significantly altering the amphipathic properties of the peptide, and hence without significantly deleteriously affecting the antimicrobial properties of the peptide. Thus, also contemplated for use with the present methods are active forms of the protegrin peptides which are composed of one or more D-amino acids. It will be appreciated that peptides composed wholly of D-amino acids can provide significant advantages under certain conditions owing to their resistance to proteases.

The ability to design active protegrin peptides composed partially or wholly of D-amino acids is well within the capabilities of those having skill in the art. Additional guidance can be found, for example, in WO 98/03192 and application Serial No.08/685,589, filed July 24, 1996.

When D-amino acids are used, preferably all of the amino acids which compose the peptide are D-amino acids. It is a well-known phenomenon that peptides composed of D-amino acids fold into a structure that is the mirror image of the structure adopted by the corresponding L-peptide. Since, as discussed above, it is believed that the antimicrobial activity of the protegrin peptides is due in large part to their amphipathic structure, protegrins

composed entirely of D-amino acids retain this amphipathic structure and therefore retain significant antimicrobial activity. Particularly preferred D-peptides for use in the methods of the invention are the D-isomers of protegrin peptides PG-1, IB-367, IB-315, IB-482 and IB-734, including the various N- and/or C-terminal modified forms and disulfide bridged forms discussed *supra*.

The active protegrin peptides described herein can be prepared in the form of their pharmaceutically acceptable salts. Pharmaceutically acceptable salts are salts which substantially retain the desired biological activity of the parent protegrin peptide and which do not impart undesired toxicological effects. Examples of such salts are acid addition salts formed with inorganic acids (*e.g.*, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like) or organic acids (*e.g.*, acetic acid, trifluoroacetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, *p*-toluenesulfonic acid, naphthalenedisulfide acid, polygalacturonic acid and the like); and salts derived from organic bases (*e.g.*, dicyclohexyl amine, and N-methyl-D-glucamine). A particularly preferred pharmaceutical salt for use in the present methods is the hydrochloride salt.

5.3.2 METHODS OF PREPARATION

Certain protegrin peptides useful in the methods of the invention, such as naturally occurring protegrins PG-1 through PG-5, can be isolated from porcine leukocytes as described in U.S. Patent No. 5,464,823 and WO 96/37508. All of the protegrin peptides can be chemically synthesized using standard art-known techniques. The N- and/or C-terminus can be derivatized, again using conventional chemical techniques. The compounds of the invention may

optionally contain a (C_1-C_8) acyl group, preferably an acetyl group at the amino terminus. Methods for acetylating or, more generally, acylating, the free amino group at the N-terminus are generally known in the art; in addition, the N-terminal amino acid may be supplied in the synthesis in acylated form.

The carboxyl terminus may be esterified using alcohols of the formula ROH wherein R is (C_1-C_8) alkyl. Similarly, the carboxyl terminus may be amidated so as to have the formula $-C(O)NH_2$, $-C(O)NHR$, or $-C(O)NRR$, wherein each R is independently (C_1-C_8) alkyl. Techniques for esterification and amidation are all standard organic chemical techniques.

Formation of disulfide linkages, if desired, is conducted in the presence of mild oxidizing agents. Chemical oxidizing agents may be used, or the compounds may simply be exposed to the oxygen of the air to effect these linkages. Various methods are known in the art. Processes useful for disulfide bond formation have been described by Tam *et al.*, 1979, Synthesis 955-957; Stewart *et al.*, 1984, Solid Phase Peptide Synthesis, 2d Ed. Pierce Chemical Company Rockford, IL; Ahmed *et al.*, 1975, *J Biol Chem* 250:8477-8482 and Pennington *et al.*, *Peptides 1990*, E. Giralt *et al.*, 1991, ESCOM Leiden, The Netherlands 164-166. An additional alternative is described by Kamber *et al.*, 1980, *Helv Chim Acta* 63:899-915. A method conducted on solid supports is described by Albericio, 1985, *Int J Pept Protein Res* 26:92-97. A particularly preferred method is solution oxidation using molecular oxygen.

Alternatively, the sulfhydryl groups of any cysteine or other sulfhydryl-containing amino acid residues can be stabilized by reacting with alkylating agents using well-known methods.

If the protegrin peptide is composed entirely of gene-encoded amino acids, or if some portion of it is so composed, the peptide or the relevant portion may also be

synthesized using recombinant DNA techniques. The DNA encoding the peptides of the invention may itself be synthesized using commercially available equipment; codon choice can be integrated into the synthesis depending on the nature of the host.

Recombinantly produced forms of the protegrins may require subsequent derivatization to modify the N- and/or C-terminus and, depending on the isolation procedure, to effect the formation of disulfide bonds as described hereinabove. Depending on the host organism used for recombinant production and the animal source from which the protein is isolated, some or all of these conversions may already have been effected.

For recombinant production, the DNA encoding the protegrins of the invention is included in an expression system which places these coding sequences under control of a suitable promoter and other control sequences compatible with an intended host cell. Types of host cells available span almost the entire range of the plant and animal kingdoms. Thus, the protegrins of the invention could be produced in bacteria or yeast (to the extent that they can be produced in a nontoxic or refractile form or utilize resistant strains) as well as in animal cells, insect cells and plant cells. Indeed, modified plant cells can be used to regenerate plants containing the relevant expression systems so that the resulting transgenic plant is capable of self protection vis-à-vis these infective agents.

Suitable recombinant methods and expression systems will be apparent to those of skill in the art.

Alternatively, protegrin peptides composed either wholly or partially of non-encoded amino acids can be made by the biosynthetic methods described in Ellman *et al.*, 1995 Methods Enzymol 202:301-336 or Noren *et al.*, 1990, Nucl. Acids Res. 18:83-88.

5.3.3 MODES OF ADMINISTRATION AND PHARMACEUTICAL FORMULATIONS

The active protegrin peptides are administered locally to the lungs of the subject, typically by generating an aerosol comprising respirable particles of protegrin peptide which the patient orally or nasally inhales. For oral inhalation, the respirable particles may be liquid or solid, and should be of sufficiently small size to pass through the mouth and larynx without impaction upon inhalation and into the bronchi and alveoli of the lungs. In general, particles ranging from about 0.5 to 10 microns (μm) in size are orally respirable. Preferably, orally respirable compositions will contain particles less than about 5 μm, more preferably in the range of 0.5-4 μm. Since particles of larger size may be deposited in the throat and swallowed, the quantity of non-respirable particles in the aerosol should be minimized. For a discussion of suitable particle size ranges for oral inhalation, *see, e.g.*, Ansel *et al.*, 1995, Pharmaceutical Dosage Forms and Drug Delivery Systems, 6th Edition, Lea and Febiger, Philadelphia, PA.

Aerosols of liquid particles comprising the active protegrin peptides may be produced by any suitable means, such as with a nebulizer. *See, e.g.*, U.S. Patent No. 4,501,729. Nebulizers are commercially available devices which transform solutions or suspensions of the active ingredient into a therapeutic aerosol mist either by means of acceleration of a compressed gas, typically air or oxygen, through a narrow venturi orifice or by means of ultrasonic agitation. Two suitable commercially available nebulizers include those sold under the tradenames PERMANEB® (De Vilbiss) and PRONEB® (Pari). Suitable formulations for use in nebulizers comprise the active ingredient in a liquid carrier or vehicle, the active ingredient comprising up to about 10% w/v of the formulation, limited in part by solubility. For IB-367, the solution preferably comprises less than about 1% w/v of the peptide, as this peptide precipitates at higher

concentrations. The carrier or vehicle is typically water, which is preferably made isotonic with body fluids by the addition of, for example, from about 2.5 to 10 % (w/v) of a non-ionic osmolites such as mono- and/or di-saccharides. In a preferred embodiment, the carrier or vehicle is made isotonic with about 10% (w/v) of a di-saccharide or about 5% (w/v) of a mono-saccharide. Suitable mono- and di-saccharides include, for example, lactose, mannitol, sorbitol, dextrose and combinations thereof. Preferred saccharides are mannitol, sorbitol and dextrose. Preferably, the composition has a pH in the range of pH 3 to pH 6. The pH can be maintained by addition of buffers, preferably by addition of about 1 mM to 100 mM, preferably about 10 mM, of an organic acid, such as, for example, lactate or acetate.

The formulation liquid may also contain optional additives such as antioxidants, flavoring agents, volatile oils, buffering agents and surfactants.

Aerosols of solid particles comprising the active compound may likewise be produced with any solid particulate medicament aerosol generator. Aerosol generators for administering solid particulate medicaments to a subject produce particles which are respirable, as explained above, and generate a volume of aerosol containing a predetermined metered dose of a medicament at a rate suitable for human administration.

One illustrative type of solid particulate aerosol generator is an insufflator. Suitable formulations for administration by insufflation include finely comminuted powders. In the insufflator, the powder (*e.g.*, a metered dose thereof effective to carry out the treatments described herein) is contained in capsules or cartridges, typically made of gelatin or plastic, which are either pierced or opened *in situ* and the powder delivered by air drawn through the device upon inhalation or by means of a manually-operated pump.

The powder employed in the insufflator consists either solely of the active ingredient or of a powder blend comprising the active ingredient, a suitable powder diluent, such as lactose, and an optional surfactant. The active ingredient typically comprises from 0.1 to 100% w/w, and preferably from 0.3 to 30% w/w, of the formulation.

A second type of illustrative aerosol generator comprises a metered dose inhaler. Metered dose inhalers are pressurized aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in a liquified propellant. During use, these devices discharge the formulation through a valve adapted to deliver a metered volume, typically from 10 to 150 μl, to produce a fine particle spray containing the active ingredient. Suitable propellants are well-known in the art and include, *e.g.*, chlorofluorocarbon compounds such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane and mixtures thereof. Of course, propellants which do not cause damage to the ozone layer or other environmental damage are preferred. The formulation may additionally contain one or more cosolvents (*e.g.*, ethanol), surfactants (*e.g.*, oleic acid or sorbitan trioleate), antioxidants and suitable flavoring agents.

The aerosol, whether formed from solid or liquid particles, is typically produced by the aerosol generator at a rate of from about 3 to 150 liters per minute, more preferably from about 5 to 60 liters per minute. Commercially available PERMANEB® nebulizers, which deliver particles in carrier gas at a rate of about 5-8 liters per minute, generally provide good results.

5.3.4 EFFECTIVE DOSAGES

The active protegrin peptide is administered to a patient in an amount effective to provide therapeutic benefit. Of course, what constitutes therapeutic benefit depends on the condition being treated.

For the treatment of cystic fibrosis, therapeutic benefit includes the reduction or amelioration of symptoms or side-effects commonly associated with cystic fibrosis, including, for example, a reduction in the bacterial burden (CFU) of infection-causing pathogens in the airways of the patient, an improvement in lung function parameters and/or a reduction in fever or other symptoms associated with cystic fibrosis. Typically, an administered dose which provides an increase of at least about 5% in Forced Vital Capacity (FVC) or an increase of at least about 5% in Forced Expiratory Volume in one sec. (FEV) relative to untreated controls is considered to be therapeutically effective for cystic fibrosis.

For the treatment of pulmonary infections, therapeutic benefit includes the reduction or amelioration of the symptoms associated with the infection, a reduction in the lungs of the patient of the bacterial burden (CFU) of infection-causing pathogens and/or a decrease in the rate at which the pathogens proliferate. Typically, a reduction of CFU on the order of one to three log is considered to be therapeutically effective; however, even reductions on the order of one log may provide significant amelioration of symptoms, and hence therapeutic benefit.

In addition to providing therapeutic benefit to patients with pulmonary infections, the protegrin peptides are also effective when used prophylactically to prevent inception of pulmonary infections. Thus, a therapeutically effective dose also includes an amount of protegrin peptide sufficient to prevent the onset of infection, or an amount sufficient to reduce the incidence of infection in patients at high risk for developing pulmonary infections, such as patients suffering from COPD, bronchiectasis and/or cystic fibrosis and/or the patients who smoke. As the protegrin peptides are effective under conditions of high salinity and can be used frequently and over long durations without engendering resistance, therapeutic and prophylactic use to combat and/or prevent persistent or recurrent pulmonary

infections, such as those observed in patients with COPD, bronchiectasis or cystic fibrosis, is an important aspect of the present invention.

For any protegrin peptide, a therapeutically effective dose can be determined from *in vitro* tests, such as, for example, those that measure minimal inhibitory concentrations (MICs) or killing kinetics. Initial dosages can also be determined from *in vivo* animal models or from clinical dosages effective for other antimicrobial agents. For example, 100 mg of colistimethate administered by inhalation twice daily has been reported to be an effective treatment for cystic fibrosis (Jensen et al., 1987, J. Antimicr. Chemother. 19:831-838). Effective dosages for protegrin peptides can be determined based on a comparison of the MIC and kill kinetics of the particular protegrin with that of colistimethate. Additionally, animal models of chronic lung infections have been described for evaluating antimicrobial agents (*see, e.g.*, Beaulac *et al.*, 1996, Antimicrobial Agents and Chemotherapy 40: 665-669). One having ordinary skill in the art could readily optimize administration to humans based on such data. Of course, the actual amount administered will depend on such factors as the severity of the condition being treated, the mode or route of administration, the weight of the subject being treated, and other factors that will be apparent to the prescribing physician.

The protegrin peptides can be administered alone, in combination with one another, or in combination with other antimicrobial or therapeutic agents. Combinations of protegrin peptides can be administered which are specifically tailored to combat infections caused by a plurality of microorganisms. Determining suitable combinations is well within the capabilities of those having skill in the art.

For administration *via* inhalation, the aerosolized protegrin peptide is administered to the lungs of a subject

in an amount sufficient to achieve a dissolved concentration on the airway surfaces that is at least 10 times the MIC for the particular target pathogen. The actual amount administered to achieve this concentration will depend in part on the efficiency of delivery, which in turn depends in part on the sizes of the respirable particles. Inhaled dosages in the range of 0.001 to 5 mg/kg/day, preferably 0.04 to 1.2 mg/kg/day, are considered effective to combat pulmonary infections and to treat cystic fibrosis. The doses may be administered once per day or multiple times per day. In most instances, the protegrin peptide will be administered from 1-4, more preferably from 1-2 times per day. Treatment regimens will usually be carried out for several days, or even for as long as weeks or months, depending on the judgment of the prescribing physician.

The invention having been described, the following examples are intended to illustrate and not limit the invention.

EXAMPLE 1: SYNTHESIS OF PROTEGRIN PEPTIDE IB-367

1.1 SYNTHESIS OF LINEAR PEPTIDE

Linear amidated IB-367 was synthesized on Rink amide solid support resin (Bachem) using Fmoc chemistry on an automated Applied Biosystems ABI 433 peptide synthesizer (Perkin Elmer, Foster City, California) according to the manufacturer's standard protocols. Cleavage of the crude product from the resin was carried out in 10 ml of 9:1:1 TFA/EDT/Anisole for 2 hrs. at room temperature. Crude cleavage product was precipitated with 40 ml ethylether, filtered and dried.

1.2 FORMATION OF DISULFIDE LINKAGES

The crude linear peptide was dissolved in DMSO and added to 20 mM ammonium acetate, pH 7. The final concentration of peptide was about 1-8 mg/mL, the pH ranged

from 7.0-7.2 and the DMSO concentration ranged from about 5-20%. The solution was stirred overnight at room temperature, and the pH of the solution was adjusted to pH 5 with concentrated acetic acid.

The oxidized peptide was loaded onto a preparative reverse-phase HPLC column (Vydac C18, 2.2cm X 25cm, Cat. No. 218TP101522), the column was washed with buffer (10% v/v acetonitrile, 0.1% v/v TFA in water) until absorbance of the effluent (measured at 235 cm) reached baseline and the pure product was eluted at 10 mL/min. using the following buffers and gradient:

Gradient

Time (min.)	Buffer A (%)	Buffer B (%)	Gradient
0	90	10	linear
10	82	18	linear
80	68	32	linear
95	5	95	linear

Buffer A= 0.10% (v/v) aqueous TFA;
Buffer B= 0.08% (v/v) TFA in acetonitrile.

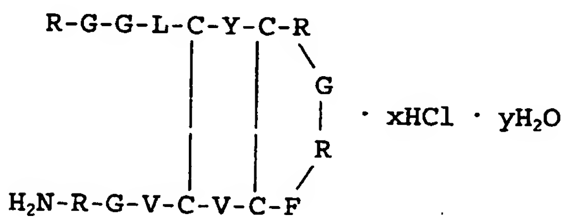
Fractions were analyzed by analytical HPLC. Fractions containing the desired disulfide-bridged peptide were pooled, the acetonitrile stripped and the resultant aqueous solution lyophilized to dryness. The sequence of the disulfide-bridged peptide was confirmed by mass spectrometry.

Protegrin peptides PG-1 (SEQ ID NO:1), IB-315 (SEQ ID NO:7), IB-482 (SEQ ID NO:8) and IB-734 (SEQ ID NO:9), which are amidated at the C-terminus with a group of the formula $-C(O)NH_2$, were synthesized, folded and purified in a manner similar to IB-367 (SEQ ID NO:6). Protegrin peptide IB-247 (SEQ ID NO:10), which is the C-terminal acid (-C(O)OH) form of peptide PG-1, was synthesized as described using either conventional Wang resin (Wang, 1973, J. Am. Chem. Soc. 95:1328) or HMBP resin (Sieber, 1987, Tetrahedron Lett.

28:6147). Folding and purification were as described for IB-367 (SEQ ID NO:6).

1.3 CONVERSION TO HYDROCHLORIDE SALT

Pure, folded IB-367 was converted to the hydrochloride salt using an anion exchange column (HCl form). The eluent was lyophilized to yield a white powder containing approximately 80 wt% active peptide. The active hydrochloride salt of IB-367 can be represented as follows:



EXAMPLE 2: PREPARATION OF INHALABLE FORMULATION

For administration by inhalation, IB-367 (hydrochloride salt) is dissolved in water containing 10 mM lactic acid (pH 4.0) and 5% (w/v) dextrose to a concentration of 10 mg/ml. 5% (w/v) sorbitol or mannitol can be used instead of dextrose. It should be noted that IB-367 hydrochloride is soluble in aqueous solutions up to a concentration of 10 mg/ml. At concentrations of 15 mg/ml and higher, a gel will form after several hours.

The solution is then sterilized by passing through a 0.22 μm filter and stored in Type-1 glass vials. For delivery, the sterile solution is aerosolized using a nebulizer with a target mass median aerodynamic diameter of 2-4μm.

EXAMPLE 3: IB-367 IS BACTERICIDAL AGAINST PATHOGENS ASSOCIATED WITH CYSTIC FIBROSIS AND OTHER RESPIRATORY INFECTIONS

3.1 EXPERIMENTAL PROTOCOL

To evaluate the effectiveness of IB-367 (in the form of the hydrochloride salt) against microorganisms found in the airways of patients with cystic fibrosis, minimum inhibitory concentrations (MICs) of IB-367 were determined against representative bacterial strains using the modified version of the NCCLS microbroth dilution method described in Steinberg et al., 1997, Antimicrob. Agents Chemother. 41:1738-1742. Conventional antimicrobial compounds were tested as controls.

3.2 RESULTS

The bactericidal activity of IB-367 against the selected pathogens is shown in Tables 2, 3, 4 and 5, below. IB-367 exhibited significant antimicrobial activity against all of the strains except *B. cepacia*. With respect to this strain, colistimethate sodium and tobramycin also failed to exhibit significant antimicrobial activity.

TABLE 2

THE MINIMUM INHIBITORY CONCENTRATION (MIC, μg/mL) OF IB-367 AGAINST MICROORGANISMS ASSOCIATED WITH CYSTIC FIBROSIS SPUTUM

				MIC (μg/ml)				
					Aminoglycosides		Polymyxins	
Genus	Species	Type	No.†	IB-367	Gentamicin	Tobramycin	Polymyxin B	Colistin*
Alcaligenes	*xylosoxidans*	--	9	2 - 16	NT	>64	NT	4 - >64
Burkholderia	*cepacia*	--	2	>64	NT	>64	NT	>64
Flavobacterium		--	2	16 - 32	NT	>128	NT	>64
Haemophilus	*influenzae*	--	3	1 - 8	NT	1	NT	NT
Pseudomonas	*aeruginosa*	mucoid	23	0.5 - 8	0.5 - 128	0.125 - 64	<0.06 - 0.5	0.5 - 4
Pseudomonas	*aeruginosa*	rough	16	0.25 - 16	2 - >64	0.5 - >64	0.25 - 1	1 - 16
Pseudomonas	*aeruginosa*	smooth	12	0.5 - 16	2 - >128	<0.06 - 128	0.25 - 32	1 - >64
NFGN	--	--	3	2 - 32	4 - 64	2 - 32	<0.06 - 4	0.75 - >128
Staphylococcus	*aureus*	--	19	1.6 - 4	NT	0.03 - 128	NT	NT
Stenotrophomonas	*maltophilia*	--	11	0.25 - 6	NT	16 > 128	NT	64

NT: not tested
NFGN: non-fermenting Gram-negative rod; presumptive *Pseudomonas* species
* Sulfomethylated, pro-drug form (colistimethate sodium)
† No. refers to the number of strains tested.

TABLE 3

MICS (mg/ml) OF IB-367, TOBRAMYCIN, POLYMYXIN B AND COLISTIMETHATE AGAINST RESPIRATORY PATHOGENS

Genus	SPECIES	Type	Strain ID	IB-367	Tobramycin	Polymixin B	Colistimethate
Alcaligenes	*spp*		411	16	>64	>64	>64
Alcaligenes	*xylosoxidans*		409	16	>64	16	>64
Alcaligenes	*xylosoxidans*		410	2	64	0.5	4
Alcaligenes	*xylosoxidans*		418	16	>64	2	>64
Alcaligenes	*xylosoxidans*		422	8	64	2	64
Alcaligenes	*xylosoxidans*		424	4	>64	0.5	8
Alcaligenes	*xylosoxidans*		451	2	>64	0.5	8
Alcaligenes	*xylosoxidans*		356	2	>64	0.5	32
Alcaligenes	*xylosoxidans*		382	16	>64	2	64
Burkholderia	*cepacia*		423	>64	>64	>64	>64
Burkholderia	*cepacia*		452	>64	>64	>64	>64
Flavobacterium	*indologenes*		412	16	>64	>64	>64
Flavobacterium			334	32	>128		

Genus	SPECIES	Type	Strain ID	IB-367	Tobramycin	Polymixin B	Colistimethate
Non-Fermenting	*GN*		355	2	2	<0.06	0.75
Pseudomonas	*aeruginosa*	mucoid	324	2	1		
Pseudomonas	*aeruginosa*	mucoid	326	2	0.25	0.38	4
Pseudomonas	*aeruginosa*	mucoid	328	2	0.5	0.25	
Pseudomonas	*aeruginosa*	mucoid	335	2	2		
Pseudomonas	*aeruginosa*	mucoid	340	8	1		
Pseudomonas	*aeruginosa*	mucoid	341	3	48	0.5	
Pseudomonas	*aeruginosa*	mucoid	342	2	8		
Pseudomonas	*aeruginosa*	mucoid	350	3	0.5	0.25	3
Pseudomonas	*aeruginosa*	mucoid	354	2	4	0.125	1
Pseudomonas	*aeruginosa*	mucoid	357	2	0.125	0.25	2
Pseudomonas	*aeruginosa*	mucoid	359	0.5	0.5	0.25	0.5
Pseudomonas	*aeruginosa*	mucoid	361	2	0.25	0.125	1
Pseudomonas	*aeruginosa*	mucoid	363	2	0.25	<0.06	0.5
Pseudomonas	*aeruginosa*	mucoid	364	2	0.5	0.125	0.5
Pseudomonas	*aeruginosa*	mucoid	367	8	6	0.5	1
Pseudomonas	*aeruginosa*	mucoid	373	2	1	0.5	1
Pseudomonas	*aeruginosa*	mucoid	376	2	4	0.25	1
Pseudomonas	*aeruginosa*	mucoid	384	2	24	0.25	1

Genus	SPECIES	Type	Strain ID	IB-367	Tobramycin	Polymixin B	Colistimethate
Pseudomonas	*aeruginosa*	rough	329	4	16		
Pseudomonas	*aeruginosa*	rough	331	8	40	0.5	
Pseudomonas	*aeruginosa*	rough	336	16	12		
Pseudomonas	*aeruginosa*	rough	339	4	2	1	
Pseudomonas	*aeruginosa*	rough	345	12	0.5		
Pseudomonas	*aeruginosa*	rough	348	8	0.5	0.5	4
Pseudomonas	*aeruginosa*	rough	358	8	4	0.5	8
Pseudomonas	*aeruginosa*	rough	366	4	2	1	4
Pseudomonas	*aeruginosa*	rough	368	2	16	0.25	2
Pseudomonas	*aeruginosa*	rough	370	4	8	0.5	2
Pseudomonas	*aeruginosa*	rough	374	8	16	1	16
Pseudomonas	*aeruginosa*	rough	377	2	2	0.5	2
Pseudomonas	*aeruginosa*	rough	379	4	8	0.5	4
Pseudomonas	*aeruginosa*	rough	381	3	>64	0.75	4
Pseudomonas	*aeruginosa*	rough	387	0.25	16	0.25	1
Pseudomonas	*aeruginosa*	smooth	325	16	4	0.5	
Pseudomonas	*aeruginosa*	smooth	327	0.5	64		
Pseudomonas	*aeruginosa*	smooth	330	12	64		
Pseudomonas	*aeruginosa*	smooth	349	2	0.5	0.25	2

Genus	SPECIES	Type	Strain ID	IB-367	Tobramycin	Polymixin B	Colistimethate
Pseudomonas	*aeruginosa*	smooth	352	2	2	0.25	
Pseudomonas	*aeruginosa*	smooth	365	16	64	0.5	16
Pseudomonas	*aeruginosa*	smooth	378	8	>64	32	>64
Pseudomonas	*aeruginosa*		385	2	48	0.25	0.75
Pseudomonas	*aeruginosa*		93-1631	2	ND		
Pseudomonas	*aeruginosa*		93-1623	2	ND		
Pseudomonas	*aeruginosa*		DO82	8	ND		
Pseudomonas	*aeruginosa*		K46	7	0.25		
Pseudomonas	*aeruginosa*		K95	7	0.25		
Pseudomonas	*aeruginosa*		K129	3	0.25		
Pseudomonas	*aeruginosa*		K228	3	0.25		
Pseudomonas	*aeruginosa*		K319	3	0.25		
Pseudomonas	*aeruginosa*		K385	3	0.25		
Pseudomonas	*aeruginosa*		K402	7	0.25		
Pseudomonas	*aeruginosa*		K497	7	0.03		
Pseudomonas	*aeruginosa*		K546	7	0.5		
Pseudomonas	*aeruginosa*		K579	7	0.5		
Pseudomonas	*aeruginosa*		K602	7	0.25		
Pseudomonas	*aeruginosa*		K636	7	0.5		

Genus	SPECIES	Type	Strain ID	IB-367	Tobramycin	Polymixin B	Colistimethate
Pseudomonas	*aeruginosa*		28	0.5	0.42	0.5	8
Pseudomonas	*aeruginosa*	mucoid	54	2	0.5	0.5	4
Pseudomonas	*aeruginosa*		167	1.1	0.38		
Pseudomonas	*species*	mucoid	343	2	0.5		
Pseudomonas	*species*	mucoid	369	2	4	0.5	1
Pseudomonas	*species*	NFGN	344	2	2		
Pseudomonas	*species*	Rough	360	16	16	0.5	8
Pseudomonas	*species*	smooth	351	1	64	0.25	
Pseudomonas	*species*	smooth	372	1	<0.06	0.125	1
Pseudomonas	*species*	smooth	375	8	32	2	>64
Stenotrophomonas			319	1	16		
Stenotrophomonas			320	1	32		
Stenotrophomonas			321	1	>128		
Stenotrophomonas			322	0.25	128		
Stenotrophomonas			323	1	>128		
Stenotrophomonas			332	2	128		
Stenotrophomonas			333	4	>128		

Genus	SPECIES	Type	Strain ID	IB-367	Tobramycin	Polymixin B	Colistimethate
Stenotrophomonas			337	2	48		
Stenotrophomonas			338	0.75	24		
Stenotrophomonas			346	2	>64	0.5	>64
Stenotrophomonas			362	6	>64	0.5	64

TABLE 4

MICS (mg/ml) OF IB-367 AND TOBRAMYCIN AGAINST *S. AUREUS*

Genus	SPECIES	Strain	IB-367	Tobramycin
Staphylococcus	*aureus*	K18	3	2
Staphylococcus	*aureus*	K49	1.6	0.125
Staphylococcus	*aureus*	K105	3	0.125
Staphylococcus	*aureus*	K123	3	0.125
Staphylococcus	*aureus*	K222	3	128
Staphylococcus	*aureus*	K283	1.6	0.03
Staphylococcus	*aureus*	K336	3	0.125
Staphylococcus	*aureus*	K380	3	0.125
Staphylococcus	*aureus*	K445	3	128
Staphylococcus	*aureus*	K559	1.6	0.25
Staphylococcus	*aureus*	K613	1.6	0.125
Staphylococcus	*aureus*	K634	3	0.125
Staphylococcus	*aureus*	SD22068	4	ND
Staphylococcus	*aureus*	SD24076	4	ND
Staphylococcus	*aureus*	CO18292	2	ND
Staphylococcus	*aureus*	CO32205	2	ND
Staphylococcus	*aureus*	ATCC 33591	4	ND
Staphylococcus	*aureus*	ATCC 29213	3.3	0.125

TABLE 5

MICs (μg/ml) OF IB-367, TOBRAMYCIN, TMP:SMX, AND CEFUROXIME AGAINST RESPIRATORY PATHOGENS

Genus	SPECIES	Strain	IB-367	Tobramycin	TMP:SMX*	Cefuroxime
Haemophilus	*influenzae*	113	1.3		0.5	2.1
Haemophilus	*influenzae*	427	4		0.25	0.5
Haemophilus	*influenzae*	428	4		>64	1.7
Haemophilus	*influenzae*	429	1.7		<0.06	0.5
Haemophilus	*influenzae*	431	5.3		0.5	0.25

Genus	*SPECIES*	Strain	IB-367	Tobramycin	TMP:SMX*	Cefuroxime
Haemophilus	*influenzae*	432	2		0.125	3.3
Haemophilus	*influenzae*	433	2		0.125	0.5
Haemophilus	*influenzae*	434	0.5		>32	0.125
Haemophilus	*influenzae*	435	1.7		0.5	1.7
Haemophilus	*influenzae*	453	4		0.125	0.5
Haemophilus	*influenzae*	458	4		16	0.5
Haemophilus	sp	K5	0.4	0.25		
Haemophilus	sp	K206	0.4	0.25		
Haemophilus	sp	K247	0.83	0.25		
Haemophilus	sp	K477	0.4	0.25		
Haemophilus	sp	K675	0.83	0.5		
Haemophilus	sp	K248	0.4	0.5		
Haemophilus	sp	K450	0.4	0.25		
Haemophilus	sp	B678	1.6	0.5		
Haemophilus	sp	B682	0.2	0.25		
Haemophilus	sp	B687	0.4	0.5		
Haemophilus	sp	B691	0.2	0.25		
Haemophilus	sp	B692	0.83	0.25		
Moraxella	*catarrhalis*	131	1.8		0.7	0.8
Moraxella	*catarrhalis*	437	0.25		1	2
Moraxella	*catarrhalis*	438	0.167		4	2
Moraxella	*catarrhalis*	439	<0.06		1	0.5
Moraxella	*catarrhalis*	440	0.25		3	0.7
Moraxella	*catarrhalis*	441	0.125		1	1
Moraxella	*catarrhalis*	442	0.06		1	0.25
Moraxella	*catarrhalis*	443	0.125		2	1
Moraxella	*catarrhalis*	444	0.125		2	2
Moraxella	*catarrhalis*	445	0.125		0.5	1
Moraxella	*catarrhalis*	446	1.6		2	2

Genus	*SPECIES*	Strain	IB-367	Tobramycin	TMP:SMX*	Cefuroxime
Moraxella	*catarrhalis*	467	0.125		1	1
Moraxella	*catarrhalis*	475	0.125		1	1
Moraxella	*catarrhalis*	476	0.125		1	1
Moraxella	*catarrhalis*	477	<0.06		2	2
Moraxella	*catarrhalis*	478	0.208		2	1
Moraxella	*catarrhalis*	479	<0.06		1	1
Moraxella	*catarrhalis*	480	<0.06		1	1
Moraxella	*catarrhalis*	481	0.125		0.5	1
Moraxella	*catarrhalis*	482	0.125		2	0.5
Moraxella	*catarrhalis*	483	0.125		2	1
Moraxella	*catarrhalis*	484	0.125		2	0.5
Moraxella	*catarrhalis*	507	0.25		1	2
Moraxella	*catarrhalis*	508	<0.06		2	0.5
Moraxella	sp	K49	0.4	0.06		
Moraxella	sp	K77	0.4	0.06		
Moraxella	sp	K195	0.4	0.06		
Moraxella	sp	K225	0.4	0.06		
Moraxella	sp	K237	0.4	0.125		
Moraxella	sp	K290	0.4	0.125		
Moraxella	sp	K426	0.2	0.125		
Moraxella	sp	K441	0.4	0.125		
Moraxella	sp	K484	0.4	0.06		
Moraxella	sp	K599	0.4	0.125		
Moraxella	sp	K639	0.2	0.06		
Moraxella	sp	K660	0.83	0.125		
Streptococcus	*pneumoniae*	127	9.2		2.3	0.26
Streptococcus	*pneumoniae*	447	10		1.2	0.4
Streptococcus	*pneumoniae*	448	7.3		0.4	0.15
Streptococcus	*pneumoniae*	449	8.6		2	<0.03

Genus	*SPECIES*	Strain	IB-367	Tobramycin	TMP:SMX*	Cefuroxime
Streptococcus	*pneumoniae*	450	12		0.8	<0.03
Streptococcus	*pneumoniae*	454	10		2	<0.03
Streptococcus	*pneumoniae*	455	8		>32	3
Streptococcus	*pneumoniae*	456	13.3		32	8
Streptococcus	*pneumoniae*	457	8		32	
Streptococcus	*pneumoniae*	468	4		1	<0.03
Streptococcus	*pneumoniae*	469	4		1	<0.03
Streptococcus	*pneumoniae*	470	16		0.5	0.125
Streptococcus	*pneumoniae*	471	16		>32	8
Streptococcus	*pneumoniae*	495	16		1	0.06
Streptococcus	*pneumoniae*	496	12		1	<0.03
Streptococcus	*pneumoniae*	497	16		>32	0.25
Streptococcus	*pneumoniae*	498	16		2	4
Streptococcus	*pneumoniae*	499	10.5		2	0.4
Streptococcus	*pneumoniae*	500	8		>32	0.4
Streptococcus	*pneumoniae*	501	8		32	10
Streptococcus	*pneumoniae*	502	16		2	<0.03
Streptococcus	*pneumoniae*	503	21.3		8	<0.03
Streptococcus	*pneumoniae*	504	8		1	<0.03
Streptococcus	*pneumoniae*	505	8		16	<0.03
Streptococcus	*pneumoniae*	506	5		16	0.125
Streptococcus	*pneumoniae*	509	9.3		2.5	<0.03
Streptococcus	*pneumoniae*	510	16		2	0.06
Streptococcus	*pneumoniae*	511	8		1.5	<0.03
Streptococcus	*pneumoniae*	512	8		2	<0.03
Streptococcus	*pneumoniae*	513	12		>32	1
Streptococcus	*pneumoniae*	514	12		3	<0.03
Streptococcus	*pneumoniae*	520	8		>32	8
Streptococcus	*pneumoniae*	521	8		>32	4
Streptococcus	*pneumoniae*	522	8		32	8
Streptococcus	*pneumoniae*	523	4		32	0.5

Genus	SPECIES	Strain	IB-367	Tobramycin	TMP:SMX*	Cefuroxime
Streptococcus	*pneumoniae*	524	4		4	16
Streptococcus	*pneumoniae*	525	4		32	32
Streptococcus	*pneumoniae*	203	8	32		
Streptococcus	*pneumoniae*	204	2	10.67		
Streptococcus	*pneumoniae*	205	2	8		
Streptococcus	*pneumoniae*	206	2	8		
Streptococcus	*pneumoniae*	264	8	32		
Streptococcus	*pneumoniae*	SD23962	2	ND		
Streptococcus	*pneumoniae*	D157	2	ND		
Streptococcus	*pneumoniae*	SD24945	2	ND		
Streptococcus	*pneumoniae*	K1	3	64		
Streptococcus	*pneumoniae*	K20	1.6	8		
Streptococcus	*pneumoniae*	K3	1.6	8		
Streptococcus	*pneumoniae*	K52	3	16		
Streptococcus	*pneumoniae*	K69	1.6	8		
Streptococcus	*pneumoniae*	K79	3	8		
Streptococcus	*pneumoniae*	K715	1.6	8		
Streptococcus	*pneumoniae*	K243	3	64		
Streptococcus	*pneumoniae*	K360	0.83	16		
Streptococcus	*pneumoniae*	K677	1.6	8		
Streptococcus	*pneumoniae*	K615	3	8		
Streptococcus	*pneumoniae*	K640	3	8		

* TMP:SMX is 5:1 trimethoprim:sulfamethoxazole

EXAMPLE 4: IB-367 IS RAPIDLY BACTERICIDAL AGAINST *P. AERUGINOSA* AND *S. AUREUS*

4.1 EXPERIMENTAL PROTOCOL

To compare IB-367 (in the form of a hydrochloride salt) and standard antibiotics for rate of bactericidal effect against *P. aeruginosa* and *S. aureus*, an *in vitro* time-kill experiment was performed. For experiments with

P. aeruginosa, stationary-phase *P. aeruginosa* (ATCC No. 9027) was suspended in Mueller-Hinton broth. Antimicrobial agents were then added to the suspensions at 2X the MIC (16 μg/ml colistimethate; 1 μg/ml colistin sulfate; 4 μg/ml IB-367; 1 μg/ml polymixin B; 0.5 μg/ml tobramycin) and the cultures incubated at 37°C. The number of viable organisms, based on an evaluation of the number of colony forming units (CFU) were determined at 5, 10, 15, 30, 60 and 120 min. after addition of antimicrobial agents.

For experiments with *S. aureus*, stationary-phase *S. aureus* (ATCC NO. 33591) was suspended in Mueller-Hinton broth. Antimicrobial agents were then added to the suspensions at 2X the MIC (1 μg/ml gentamycin; 1 μg/ml norfloxacin; 1 μg/ml vancomycin; 4 μg/ml IB-367) and the cultures incubated at 37°C. The number of viable organisms, based on an evaluation of the number of colony forming units (CFU) were determined at 5, 10, 15, 30, 60 and 120 min. after addition of antimicrobial agents.

4.2 <u>RESULTS</u>

The reduction in the number of CFU/ml as a function of time for each of the antimicrobial agents tested is depicted in FIGS. 1A-1C. Referring to FIGS. 1A and 1B, the results indicate that IB-367, colistin sulfate, and polymyxin B are more rapidly bactericidal towards *P. aeruginosa* than are colistimethate or tobramycin. The slower rate of killing observed with colistimethate sodium relative to colistin sulfate was attributed to a time-dependent hydrolytic activation process required for conversion of colistimethate (which is a pro-drug) to the active form of the molecule (Beveridge *et al.*, 1967, Br. J. Pharmac. Chemother. 29:125-135).

Referring to FIG. 1C, the results indicate that peptide IB-367 is significantly more rapidly bactericidal

against *S. aureus* than gentamicin, norfloxacin and vancomycin.

EXAMPLE 5: IB-367 KILLS *P. AERUGINOSA* IN BALF MORE RAPIDLY THAN CONVENTIONAL ANTIBIOTICS

5.1 EXPERIMENTAL PROTOCOL

Protegrin peptide IB-367 (hydrochloride salt) was compared to standard antimicrobial agents for its ability to kill *P. aeruginosa* added to bronchoalveolar lavage fluid (BALF) from a cystic fibrosis patient. *P. aeruginosa* (ATCC No. 9027) was added to previously frozen BALF to yield a density of about $1x10^8$ CFU/ml. IB-367 or standard antibiotics were added at a concentration of 100 μg/ml. Samples were incubated at 37°C and the number of viable organisms determined at 30, 120 and 240 min. by evaluating the number of CFU.

5.2 RESULTS

The results are illustrated in FIG. 2. Peptide IB-367 had significantly greater activity than colistimethate, colistin sulfate and tobramycin. The limit of detection of the experiment was 1 log CFU/ml. Separate studies (data not shown) indicated that antibiotic carryover did not interfere with accurate assessment of CFU.

EXAMPLE 6: PEPTIDE IB-367 RAPIDLY KILLS ENDOGENOUS FLORA IN CYSTIC FIBROSIS SPUTUM

6.1 EXPERIMENTAL PROTOCOL

Protegrin peptide IB-367 (hydrochloride salt) was evaluated for its ability to decrease CFU of endogenous flora in sputum pooled from patients with cystic fibrosis as compared with standard antimicrobial agents.

Sputum pooled from patients with cystic fibrosis was mixed (1:1) vigorously with an aqueous solution of mannitol:sucrose (3%:3%). In one experiment, peptide IB-367 (in buffered vehicle) was added 1:1 to the sputum

mixture to give final concentrations of 250 μg/ml, 1000 μg/ml and 4000 μg/ml. In another experiment, peptide IB-367 or antibiotics (in buffered vehicle) were added 1:1 to the sputum mixture to give a final concentration of 1000 μg/ml. All samples were mixed vigorously and incubated at 37°C. The number of viable organisms, based on an evaluation of CFU, was determined at 2, 4, 8, 16, 30, 60, 120 and 240 min. following administration of compounds.

In a third experiment, drugs were tested at concentrations of 1000 μg/ml as previously described, except that the agents were reapplied to yield an additional 1000 μg/ml dose at 120 min. after initial administration. The number of viable organisms (CFU) were determined as described.

6.2 RESULTS

The dose-dependent reduction of CFU for various concentrations of IB-367 are illustrated in FIG. 3A. The reduction of CFU for IB-367 as compared to standard antimicrobial agents is illustrated in FIG. 3B. Referring to FIG. 3A, IB-367 killed endogenous microflora rapidly (*i.e.*, within two minutes) in a concentration-dependent manner. Referring to FIG. 3B, IB-367 killed endogenous microflora to a greater extent during the first 16 minutes of exposure than did tobramycin. The reduction of CFU by IB-367 over the entire 240 min. duration was about equal to that observed for tobramycin and polymixin B. IB-367 killed endogenous flora more effectively than colistimethate over the entire 240-minute exposure period.

The results obtained upon re-addition of agents at 120 min. following initial administration are presented in FIG. 3C-3B. IB-367 showed significant improvement in kill kinetics following re-addition, as compared with conventional antibiotics, indicating that improved efficacy can be achieved with multiple dosing of IB-367.

EXAMPLE 7: PROTEGRIN PEPTIDES KILLS *P. AERUGINOSA* ON THE SURFACE OF CULTURED EPITHELIAL AIRWAY CELLS

7.1 EXPERIMENTAL PROTOCOL

To demonstrate the efficacy of protegrin peptides in eliminating *P. aeruginosa* on airway epithelial cells, three experiments were conducted. In one experiment, a 20 nl aliquot of *P. aeruginosa* was added to the mucosal surface of cultured Calu-3 epithelia monolayers (a human cell line derived from lung adenocarcinoma; ATCC No. HTB-55). Following a 2 hour incubation at 37°C, 50 µg/ml or 200 µg/ml IB-367 hydrochloride salt (in physiological saline supplemented with 0.1% albumin and 1% Luria broth) was added to the mucosal surface. Control monolayers received supplemented saline without IB-367. Following addition of compounds, the plates were incubated for 1 hour at 37°C and the viable bacteria were recovered from the mucosal surface and enumerated by plating onto agar.

In another experiment, Calu-3 monolayers inoculated with approx. 214 ± 19 CFU *P. aeruginosa* were treated with either 20 or 200 µg/ml of IB-367 (hycrochloride salt), IB-247 (TFA salt), IB-482 (TFA salt), IB-315 (TFA salt), colistimethate or tobramycin. Control epithelial monolayers received supplemented saline without antimicrobial agents.

In a third experiment, *P. aeruginosa*-inoculated Calu-3 monolayers were treated with either 0, 20, 50 or 200 µg/ml peptide IB-367 or IB-734.

7.2 RESULTS

The number of *P. aeruginosa* CFU recovered from epithelial monolayers treated with various concentrations of IB-367 are provided in TABLE 6, below. IB-367 exhibited a dose-dependent reduction in recovered *P. aeruginosa* CFU when added to monolayers.

TABLE 6

RECOVERY OF *P. AERUGINOSA* FROM CALU-3 EPITHELIAL MONOLAYERS TREATED WITH IB-367

IB-367 (μg/ml)	Number of CFU (Mean ± S.D.)
0	2100 ± 400
50	1900 ± 225
200	300 ± 100

The reductions in *P. aeruginosa* CFU following treatment with the various protegrins and conventional antibodies are presented in TABLE 7, below.

TABLE 7

ELIMINATION OF *P. AERUGINOSA* FROM CALU-3 EPITHELIAL MONOLAYERS TREATED WITH PROTEGRIN PEPTIDES

Compound	μg/ml	CFU (Mean ± S.D.)
Saline	0	305 ± 3
IB-367	20	180 ± 80
	200	0 ± 0
IB-247	20	272 ± 143
	200	105 ± 105
IB-482	20	111 ± 47
	200	1 ± 1
IB-315	20	117 ± 47
	200	1 ± 1
Colistimethate	20	8 ± 2
	200	0 ± 0
Tobramycin	20	0 ± 0
	200	0 ± 0

The reduction in *P. aeruginosa* CFU following treatment with IB-367 or IB-734 are provided in FIG. 5. Both

peptides were bactericidal against *P. aeruginosa*, with peptide IB-367 exhibiting antimicrobial activity at a concentration of 200 μg/ml. Peptide IB-734 exhibited significant antimicrobial activity at concentrations as low as 20-50 μg/ml.

EXAMPLE 8: IB-367 KILLS TOBRAMYCIN-RESISTANT *P. AERUGINOSA* ON THE SURFACE OF CULTURED EPITHELIAL AIRWAY CELLS

8.1 EXPERIMENTAL PROTOCOL

To demonstrate the ability of IB-367 (hydrochloride salt) to eliminate tobramycin-resistant *P. aeruginosa* from epithelial airway cells, a Calu-3 monolayer (ATCC No. HTB-55) was inoculated with a strain of tobramycin-resistant *P. aeruginosa* obtained from a patient with cystic fibrosis (tobramycin MIC = 64 μg/ml). Supplemented saline containing either 200 μg/ml tobramycin or IB-367 was added and the number of viable organisms recovered and enumerated as previously described. Control epithelial monolayers received supplemental saline without antimicrobial agents.

8.2 RESULTS

The recovery of tobramycin-resistant *P. aeruginosa* CFU from Calu-3 epithelial monolayers treated with tobramycin or IB-367 are provided in TABLE 8, below. IB-367 showed a markedly greater killing activity than tobramycin.

TABLE 8

RECOVERY OF TOBRAMYCIN-RESISTANT *P. AERUGINOSA* FROM CALU-3 EPITHELIAL MONOLAYERS TREATED WITH IB-367 OR TOBRAMYCIN

Treatment	Number of CFU (Mean ± S.D.)
Saline	600 ± 10

200 $\mu g/ml$ tobramycin	600 ± 50
200 $\mu g/ml$ IB-367	180 ± 75

EXAMPLE 9: IB-367 IS ACTIVE UNDER CONDITIONS OF HIGH SALINITY

9.1 EXPERIMENTAL PROTOCOL

To demonstrate the antimicrobial activity of IB-367 (hycrochloride salt) under the conditions of high salinity observed in the airways of cystic fibrosis patients, the MICs of IB-367, tobramycin and colistimethate sulfate against *P. aeruginosa* and *S. aureus* in 0 mM NaCl and 182 mM NaCl were determined as previously described.

9.2 RESULTS

The MICs of the various agents are provided in TABLE 9, below:

TABLE 9

MICs OF IB-367, TOBRAMYCIN AND COLISTIMETHATE AS A FUNCTION OF NaCl CONCENTRATION

Compound	MIC ($\mu g/ml$)			
	P. aeruginosa		*S. aureus*	
	0 mM	182 mM	0 mM	182 mM
IB-367	1.00	4.00	2.00	8.00
Colistimethate	2.00	2.00	NT	NT
Tobramycin	0.25	0.50	>16	>16

The MIC of IB-367 was essentially unaffected by 182 mM NaCl, demonstrating the efficacy of IB-367 under the conditions observed in the lungs of cystic fibrosis patients. The bactericidal activity of protegrin peptide PG-1 (IB-200; SEQ ID NO. 1) against *K. pneumonia,*

S. aureus and *E. faecium* is also unaffected by physiologic concentrations (100 mM) of NaCl (data not shown).

EXAMPLE 10: PEPTIDE IB-367 ENGENDERS LESS RESISTANCE THAN CONVENTIONAL ANTIBIOTICS

10.1 EXPERIMENTAL PROTOCOL

To demonstrate the low frequency of resistance induced by IB-367 (hydrochloride salt) as compared with other antibiotics, *P. aeruginosa* (ATCC No. 9027) and *S. aureus* (ATCC 33591) were repeatedly sub-cultured after 5 days incubation in the presence of norfloxacin, polymixin B or IB-367 (for *P. aeruginosa*) and norfloxacin, vancomycin or IB-367 (for *S. aureus*) (at a concentration of one-half the MIC of the respective agent). Following nine serial transfers, the MIC values of the respective antimicrobial agents were determined.

10.2 RESULTS

The results are shown in FIGS. 4A and 4B. Referring to FIG. 4A, IB-367 induced virtually no resistance in *P. aeruginosa*. After nine serial transfers, the MICs for polymixin B and IB-367 were relatively unaffected, increasing only 2-fold and 1-2 fold, respectively. In contrast, the MICs for tobramycin increased 160-fold.

Referring to FIG. 4B, IB-367 also induced virtually no resistance in *S. aureus*. After nine serial transfers, the MICs for IB-367 and vancomycin increased by only four-fold and two-fold, respectively. In contrast, the MICs for norfloxacin increased by 320-fold.

EXAMPLE 11: PEPTIDE IB-367 EXHIBITS A LOW RATE OF CROSS-RESISTANCE WITH CONVENTIONAL ANTIBIOTICS

11.1 EXPERIMENTAL PROTOCOL

To demonstrate the low rate of cross-resistance between aminoglycosides and IB-367 (hydrochloride salt), *P aeruginosa* (ATCC No. 9027) was sub-cultured daily in the presence of tobramycin (at a concentration of one-half times the MIC) to select for tobramycin-resistant strains. Tobramycin-resistant strains were then tested for susceptibility to gentamycin, polymixin B and IB-367.

11.2 RESULTS

The results of the cross-resistance experiments are provided in TABLE 10, below.

TABLE 10

SELECTION OF TOBRAMYCIN RESISTANCE DOES NOT RESULT IN IB-367 OR POLYMIXIN B RESISTANCE

Strain	Selection	MIC (μg/mL)			
		IB-367	Tobramycin	Gentamicin	Polymyxin B
P. aeruginosa (strain 028)	none	2	0.5	0.5	0.5
	Tobramycin	2	32	107	0.5
	MIC_F/MIC_I	1	64	214	1
P. aeruginosa 97 (mucoid clinical isolate)	none	4	0.5	0.5	0.5
	Tobramycin	2	16	32	0.5
	MIC_F/MIC_I	2	32	16	1

MIC_F/MIC_I: ratio of final MIC after 14th transfer to initial MIC.

Generation of cross-resistance between aminoglycoside tobramycin and IB-367 was extremely low. At the end of 14 days, the MIC for tobramycin increased 32-64 fold. When this population of tobramycin-resistant bacteria was tested for susceptibility to gentamicin, polymixin B and IB-367, cross-resistance to gentamicin was observed, while susceptibility of IB-367 and polymixin B remained unchanged.

EXAMPLE 12: EFFICACY IN RATS

To demonstrate the efficacy of protegrin peptides in treating pulmonary infections in animals, approximately $5x10^4$ CFU of *P. aeruginosa* (in cystein trypticase agar) having increased virulence towards rats (strain ATCC No. 29260) is administered to Sprague-Dawley rats by tracheal instillation. After infection, the rats are treated once or at multiple times with 1 to 10 mg/ml protegrin peptide solution (specifically one of the preferred protegrin peptides such as peptide IB-367 hydrochloride salt) *via* intratracheal instillation or as a respirable aerosol. At least 4 hours after the completion of treatment, the rats are euthanized and the lungs asceptically removed and homogenized in phosphate-buffered saline. The homegenates are spread onto blood agar plates for enumeration of CFU.

What Is Claimed Is:

1. A method of treating or preventing a pulmonary infection, said method comprising the step of administering to a subject in need thereof an effective amount of a protegrin peptide, or a pharmaceutically acceptable salt thereof.

2. The method of Claim 1, wherein said pulmonary infection is caused by *P. aeruginosa*, *S. aureus*, *H. influenza* or *S. pneumoniae*.

3. The method of Claim 1, wherein said pulmonary infection is caused by an antibiotic-resistant strain of bacteria.

4. The method of Claim 3, wherein said antibiotic-resistant strain of bacteria is selected from the group consisting of MRSA, TRPA and PRSP.

5. The method of Claim 1, wherein said protegin peptide has the formula:

$$Z_1\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_4\text{-}X_5\text{-}C_6\text{-}X_7\text{-}C_8\text{-}X_9\text{-}X_{10}\text{-}X_{11}\text{-}X_{12}\text{-}C_{13}\text{-}X_{14}\text{-}C_{15}\text{-}X_{16}\text{-}X_{17}\text{-}X_{18}\text{-}Z_2$$

or a pharmaceutically acceptable salt thereof, wherein:

X_1 is a basic amino acid or absent;

X_2 is a hydrophobic amino acid or absent;

X_3 is a hydrophobic amino acid or absent;

X_4 is a basic amino acid or absent;

X_5 is an aliphatic amino acid;

each of C_6, C_8, C_{13} and C_{15} is independently selected from the group consisting of a cysteine-like amino acid and a polar amino acid;

X_7 is an aromatic amino acid;

X_9 is a basic amino acid;

X_{10} is a basic amino acid or a helix-breaking amino acid;

X_{11} is a basic amino acid;

X_{12} is an aromatic amino acid;

X_{14} is an aliphatic amino acid;

X_{16} is an aliphatic amino acid;

X_{17} is an aliphatic amino acid or absent;

X_{18} is a basic amino acid or absent;

Z_1 is R-C(O)-NH- or H_2N-;

Z_2 is -C(O)OR or -C(O)NRR;

each R is independently selected from the group consisting of -H and (C_1-C_8) alkyl; and

each "-" between residues X_n and C_n is independently selected from the group consisting of amide, substituted amide, an isostere of amide and a peptidomimetic.

6. The method of Claim 5, in which X_4 is absent.

7. The method of Claim 5, in which X_{17} and X_{18} are absent.

8. The method of Claim 5 in which X_{10} is a helix-breaking amino acid.

9. The method of Claim 5, in which X_1, X_2 and X_4 are absent.

10. The method of Claim 9, in which X_{17} and X_{18} are absent.

11. The method of Claim 5, in which X_1, X_2, X_3 and X_4 are absent.

12. The method of Claim 11, in which X_{17} and X_{18} are absent.

13. The method of Claim 5 in which X_2 is an aliphatic amino acid and X_3 is an aromatic amino acid.

14. The method of Claim 5 in which X_1, X_4, X_9, X_{10}, X_{11} and X_{18} are each Arg or Dbu.

15. The method of Claim 5 in which said peptide is selected from the group consisting of SEQ ID NO:1; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9 and the N-terminal acylated, C-terminal acid and D-enatiomeric forms thereof.

16. The method of Claim 1, in which:

X_1 is Arg, Dbu or absent;

X_2 is Gly or absent;

X_3 is Gly, Trp or absent;

X_4 is Arg, Dbu or absent;

X_5 is Leu or Cha;

each of C_6, C_8, C_{13} and C_{15} is independently selected from the group consisting of Cys, Ser and Thr;

X_7 is Tyr;

X_9 is Arg or Dbu;

X_{10} is Arg, Dbu, Gly or Pro;

X_{11} is Arg or Dbu;

X_{12} is Phe;

X_{14} is Val;

X_{16} is Val;

X_{17} is Gly or absent; and

X_{18} is Arg, Dbu or absent.

17. The method of Claim 16, in which all amino acids are in the L-configuration.

18. The method of Claim 16, in which all amino acids are in the D-configuration.

19. The method Claim 16, in which X_1 is H_2N-; X_2 is $-C(O)NH_2$ and each "-" is -C(O)NH-.

20. The method of Claim 19, in which the peptide is IB-367 (SEQ ID NO:6).

21. The method of Claim 20, wherein the peptide is in the form of a hydrochloride salt.

22. The method of Claim 1, wherein said subject has cystic fibrosis.

23. The method of Claim 1, wherein said protegrin peptide is administered to the lungs of said subject in the form of aerosolized respirable particles.

24. The method of Claim 1, wherein said protegrin peptide is administered therapeutically.

25. The method of Claim 1, wherein said protegrin peptide is administered prophylactically.

26. A method of treating cystic fibrosis, said method comprising the step of administering to a subject having cystic fibrosis an amount of a protegrin peptide, or a pharmaceutically acceptable salt thereof, effective to improve lung function or amelionate or reduce symptoms associated with cystic fibrosis.

27. The method of Claim 26, wherein said protegin peptide has the formula:

$$Z_1-X_1-X_2-X_3-X_4-X_5-C_6-X_7-C_8-X_9-X_{10}-X_{11}-X_{12}-C_{13}-X_{14}-C_{15}-X_{16}-X_{17}-X_{18}-Z_2$$

or a pharmaceutically acceptable salt thereof, wherein:

X_1 is a basic amino acid or absent;

X_2 is a hydrophobic amino acid or absent;

X_3 is a hydrophobic amino acid or absent;

X_4 is a basic amino acid or absent;

X_5 is an aliphatic amino acid;

each of C_6, C_8, C_{13} and C_{15} is independently selected from the group consisting of a cysteine-like amino acid and a polar amino acid;

X_7 is an aromatic amino acid;

X_9 is a basic amino acid;

X_{10} is a basic amino acid or a helix-breaking amino acid;

X_{11} is a basic amino acid;

X_{12} is an aromatic amino acid;

X_{14} is an aliphatic amino acid;

X_{16} is an aliphatic amino acid;

X_{17} is an aliphatic amino acid or absent;

X_{18} is a basic amino acid or absent;

Z_1 is R-C(O)-NH- or H_2N-;

Z_2 is -C(O)OR or -C(O)NRR;

each R is independently selected from the group consisting of -H and (C_1-C_8) alkyl; and

each "-" between residues X_n and C_n is independently selected from the group consisting of amide, substituted amide, an isostere of amide and a peptidomimetic.

28. The method of Claim 26 in which said peptide is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and the N-terminal acylated, C-terminal acid and D-enantiomeric forms thereof.

29. A respirable pharmaceutical composition comprising a protegrin peptide, or a pharmaceutically effective salt thereof, and a pharmaceutically acceptable diluent.

30. The composition of Claim 29 wherein said protegrin peptide is present in an amount of about 0.03 to 1 wt%.

31. The composition of Claim 29, wherein said pharmaceutically acceptable diluent is an aqueous solution comprising about 10 mM lactic acid and about 5% (w/v) saccharide.

32. The composition of Claim 31, wherein the saccharide is selected from the group consisting of dextrose, sorbitol, mannitol and combinations thereof.

33. The composition of Claim 29, wherein the protegin peptide has the formula:

$$Z_1-X_1-X_2-X_3-X_4-X_5-C_6-X_7-C_8-X_9-X_{10}-X_{11}-X_{12}-C_{13}-X_{14}-C_{15}-X_{16}-X_{17}-X_{18}-Z_2$$

or a pharmaceutically acceptable salt thereof, wherein:

X_1 is a basic amino acid or absent;

X_2 is a hydrophobic amino acid or absent;

X_3 is a hydrophobic amino acid or absent;

X_4 is a basic amino acid or absent;

X_5 is an aliphatic amino acid;

each of C_6, C_8, C_{13} and C_{15} is independently selected from the group consisting of a cysteine-like amino acid and a polar amino acid;

X_7 is an aromatic amino acid;

X_9 is a basic amino acid;

X_{10} is a basic amino acid or a helix-breaking amino acid;

X_{11} is a basic amino acid;

X_{12} is an aromatic amino acid;

X_{14} is an aliphatic amino acid;

X_{16} is an aliphatic amino acid;

X_{17} is an aliphatic amino acid or absent;

X_{18} is a basic amino acid or absent;

Z_1 is R-C(O)-NH- or H_2N-;

Z_2 is -C(O)OR or -C(O)NRR;

each R is independently selected from the group consisting of -H and (C_1-C_8) alkyl; and

each "-" between residues X_n and C_n is independently selected from the group consisting of amide, substituted amide, an isostere of amide and a peptidomimetic.

34. The composition of Claim 32 in which said peptide is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9 and the N-terminal acylated, C-terminal acid and D-enantiomeric forms thereof.

35. The composition of Claim 29 which is an aqueous solution comprising about 0.03 to 1 wt% of said peptide, about 1 to 100 mM lactic acid and about 2.5 to 10 wt% mono- or di-saccharide, and which has a pH in the range of about 3 to 6.

36. The composition of Claim 35, which is an aqueous solution comprising about 0.03 to 1 wt% of said peptide, about 10 mM lactic acid, about 5% (w/v) dextrose monophosphate and which has a pH of about 4.

37. A peptide having the formula:

$$Z_1\text{-}X_1\text{-}X_2\text{-}X_3\text{-}X_4\text{-}X_5\text{-}C_6\text{-}X_7\text{-}C_8\text{-}X_9\text{-}X_{10}\text{-}X_{11}\text{-}X_{12}\text{-}C_{13}\text{-}X_{14}\text{-}C_{15}\text{-}X_{16}\text{-}X_{17}\text{-}X_{18}\text{-}Z_2$$

or a pharmaceutically acceptable salt thereof, wherein:

X_1 is Dbu;

X_2 is Gly;

X_3 is Gly;
X_4 is Dbu;
X_5 is Leu;
X_6 is Cys;
X_7 is Tyr;
X_8 is Cys;
X_9 is Dbu;
X_{10} is Dbu;
X_{11} is Dbu;
X_{12} is Phe;
X_{13} is Cys;
X_{14} is Val;
X_{16} is Cys;
X_{17} is Gly;
X_{18} is Dbu;
Z_1 is R-C(O)-NH- or H_2N-;
Z_2 is -C(O)OR or -C(O)NRR;

each R is independently selected from the group consisting of -H and (C_1-C_8) alkyl; and

each "-" between residues X_n and C_n is independently selected from the group consisting of amide, substituted amide, an isostere of amide and a peptidomimetic.

38. The peptide of Claim 37 in which Z_1 is H_2N- and Z_2 is $-C(O)NH_2$.

39. The peptide of Claim 37 in which each "-" between residues X_n and C_n is -C(O)NH-.

40. The peptide of Claim 39 in which each amino acid is an L-amino acid.

41. The peptide of Claim 39 in which each amino acid is a D-amino acid.

42. The peptide of Claim 37 which is SEQ ID NO:9.

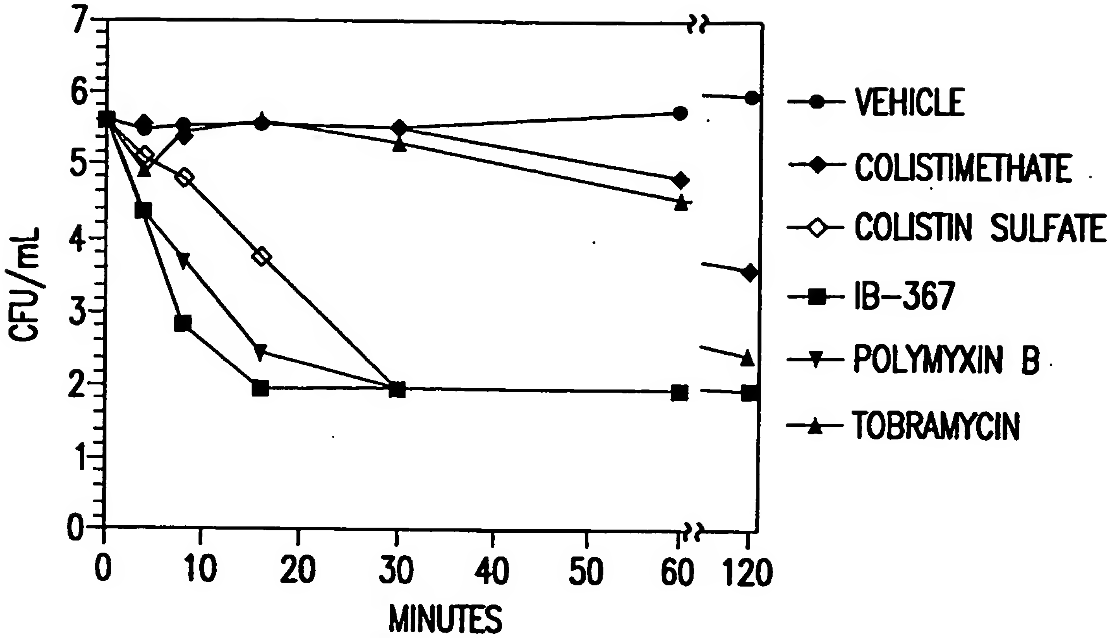

7
6
5
4
3
2
1
0
CFU/mL
0
10
20
30
40
50
60
120
MINUTES
VEHICLE
COLISTIMETHATE
COLISTIN SULFATE
IB-367
POLYMYXIN B
TOBRAMYCIN

FIG.1A

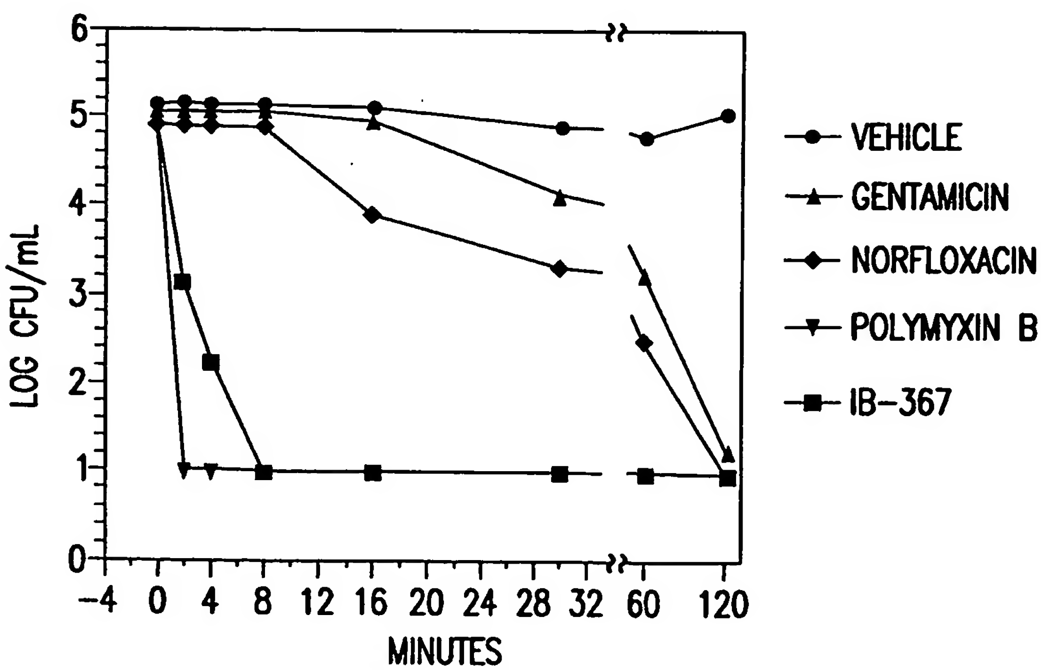

6
5
4
3
2
1
0
LOG CFU/mL
-4
0
4
8
12
16
20
24
28
32
60
120
MINUTES
VEHICLE
GENTAMICIN
NORFLOXACIN
POLYMYXIN B
IB-367

FIG.1B

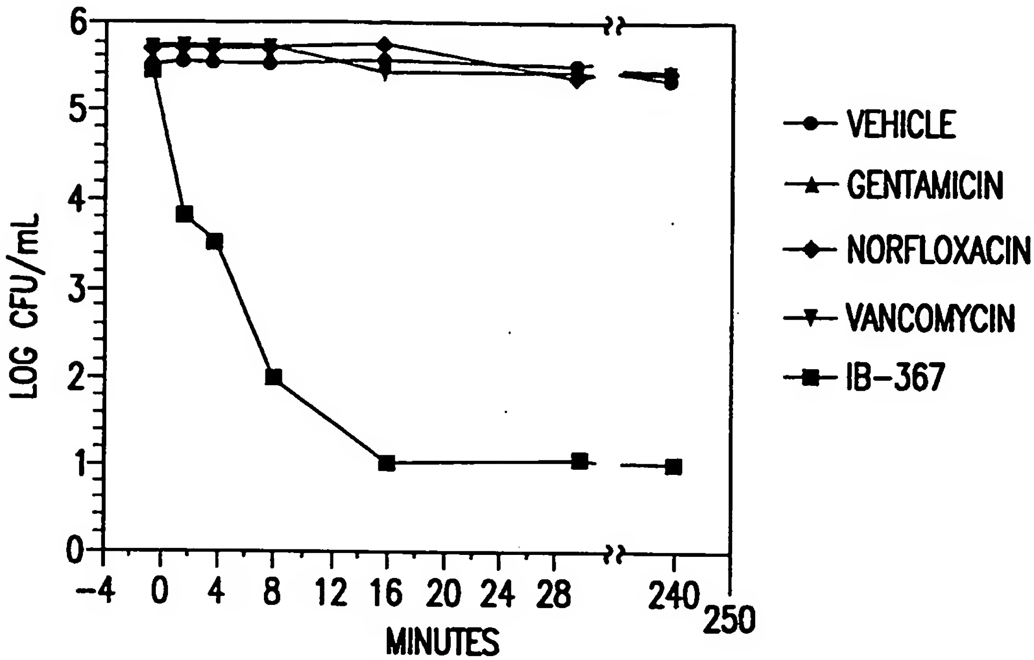

6
5
4
3
2
1
0
LOG CFU/mL
-4
0
4
8
12
16
20
24
28
240
250
MINUTES
VEHICLE
GENTAMICIN
NORFLOXACIN
VANCOMYCIN
IB-367

FIG.1C

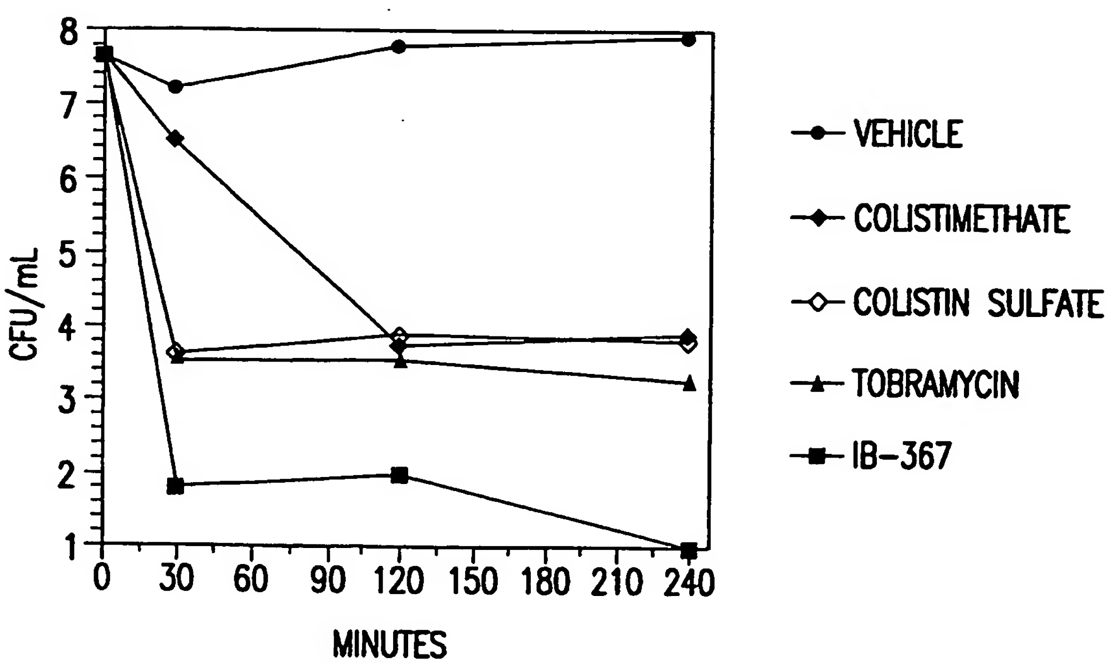

8
7
6
5
4
3
2
1
CFU/mL
0
30
60
90
120
150
180
210
240
MINUTES
VEHICLE
COLISTIMETHATE
COLISTIN SULFATE
TOBRAMYCIN
IB-367

FIG.2

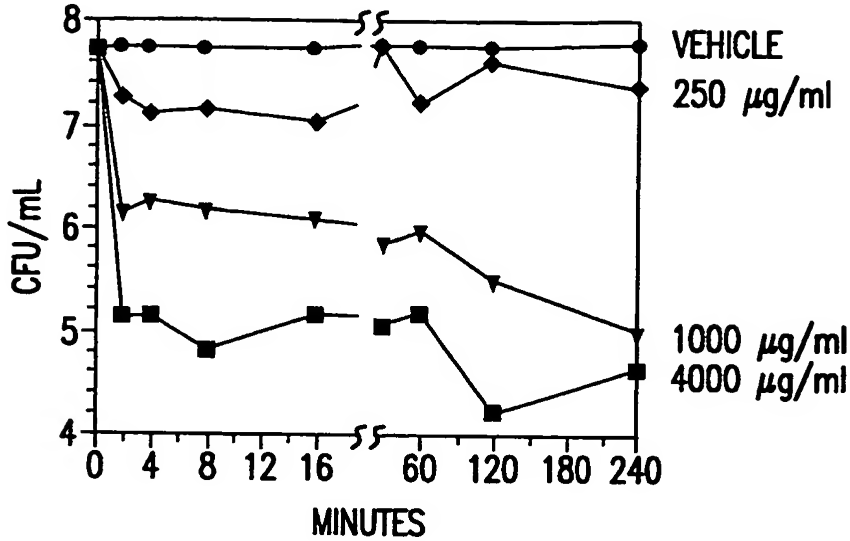

8
7
6
5
4
CFU/mL
0 4 8 12 16 60 120 180 240
MINUTES
VEHICLE
250 μg/ml
1000 μg/ml
4000 μg/ml

FIG.3A

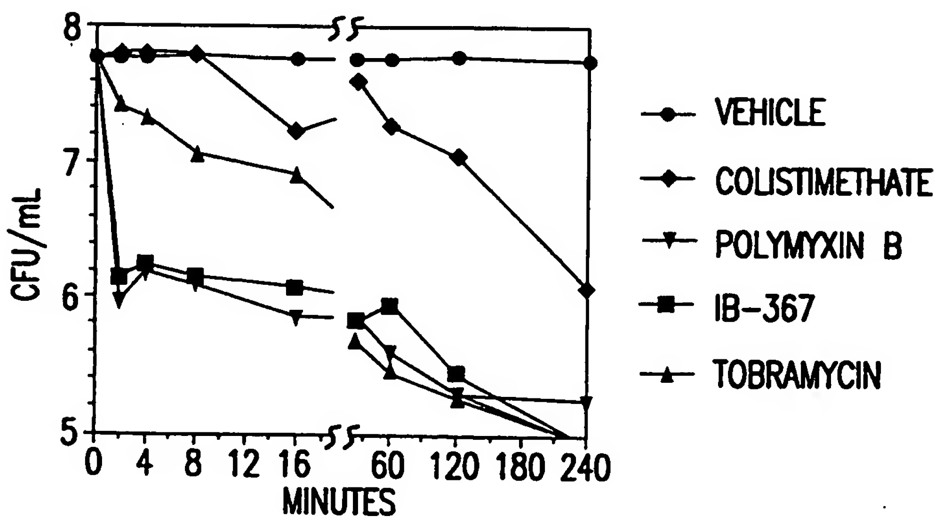
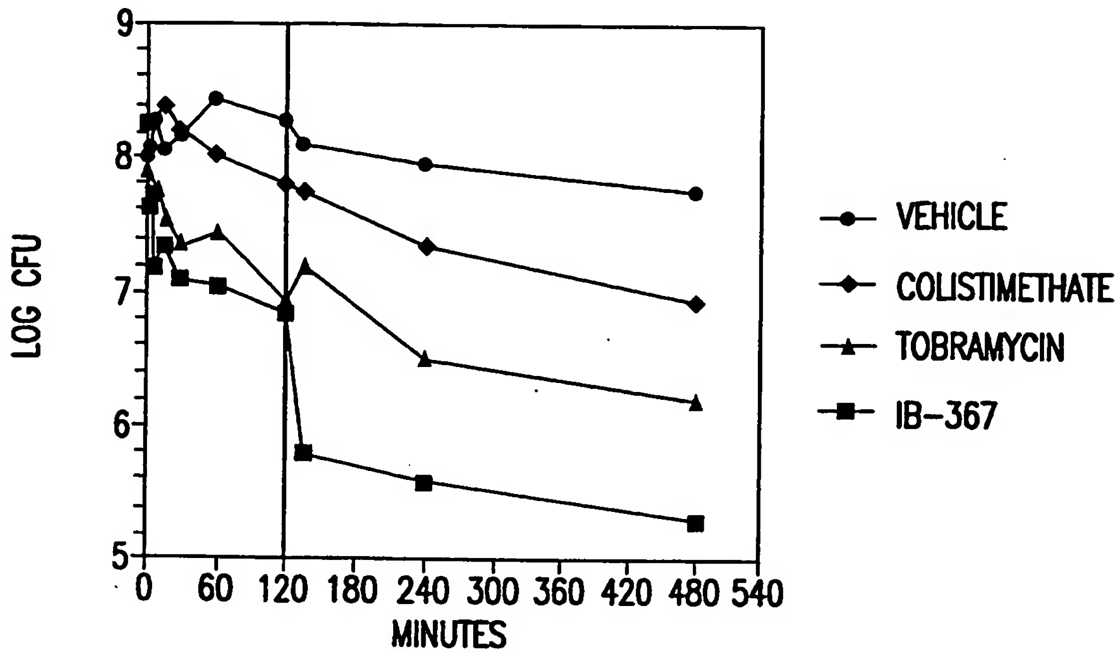

8
7
6
5
CFU/mL
0 4 8 12 16 60 120 180 240
MINUTES
VEHICLE
COLISTIMETHATE
POLYMYXIN B
IB-367
TOBRAMYCIN

FIG.3B



LOG CFU
9
8
7
6
5
0
60
120
180
240
300
360
420
480
540
MINUTES
VEHICLE
COLISTIMETHATE
TOBRAMYCIN
IB-367

FIG.3C

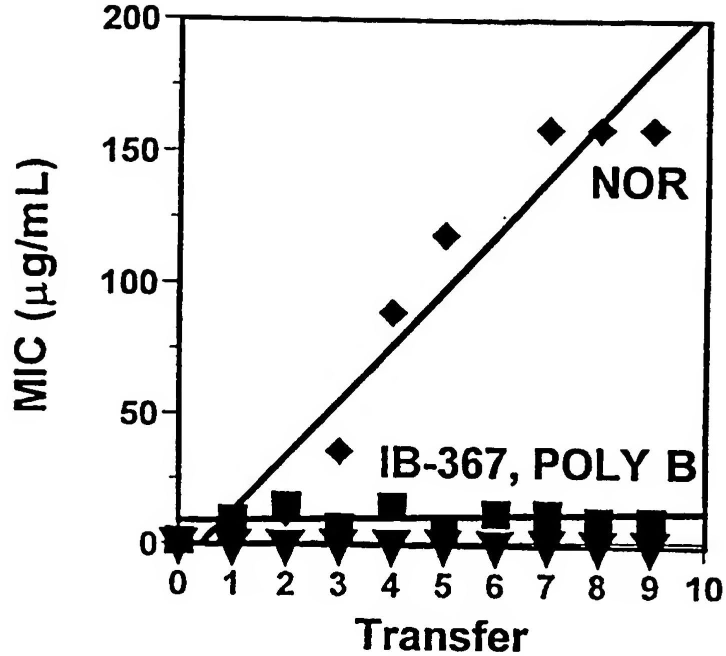

200
150
100
50
0
MIC (μg/mL)
NOR
IB-367, POLY B
0 1 2 3 4 5 6 7 8 9 10
Transfer

FIG.4A

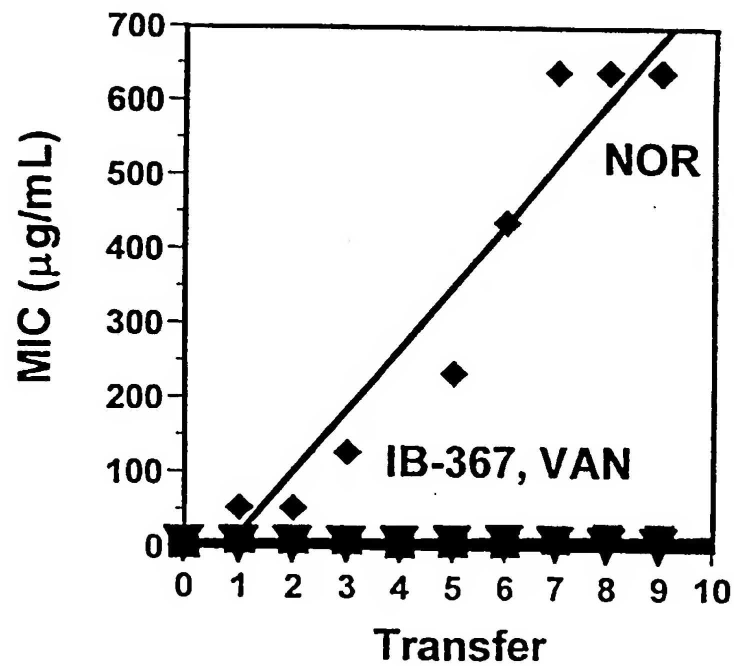

700
600
500
400
300
200
100
0
MIC (μg/mL)
NOR
IB-367, VAN
0 1 2 3 4 5 6 7 8 9 10
Transfer

FIG.4B

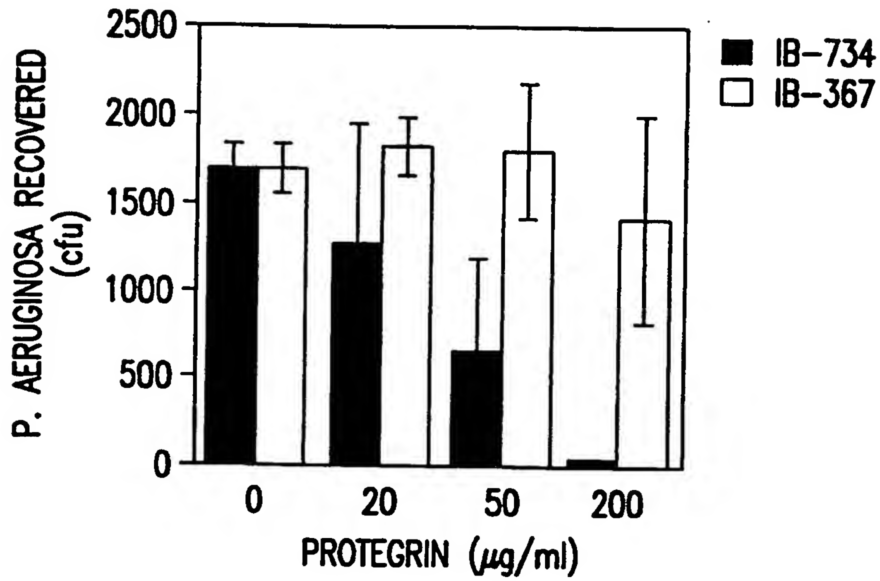

P. AERUGINOSA RECOVERED (cfu)
2500
2000
1500
1000
500
0
■ IB-734
□ IB-367
0
20
50
200
PROTEGRIN (μg/ml)

FIG.5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/16739

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/04, 38/05, 38/16; C07K 4/00, 7/00, 14/00
US CL :424/ 234.1, 243.1, 244.1, 256.1, 260.1; 514/13, 14; 530/326, 327
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/ 234.1, 243.1, 244.1, 256.1, 260.1; 514/13, 14; 530/326, 327

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, STN
search terms: protegrins, cystic fibrosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	WO 97/18826 A1 (INTRABIOTICS PHARMACEUTICALS, INC.) 29 May 1997, see entire document, especially page 43-45 and page 107, see entire document.	1-21, 24-25 ---------- 22-23, 26-36
Y	BERKOW, ROBERT. The Merck Manual of Diagnosis and Therapy. Rathway, N.J.: Merck Research Laboratories. 1992, Vol. 16, pages 2206-2210, see entire document.	22-23, 26-36

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 SEPTEMBER 1999	21 OCT 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer D. Lawrence for ANISH GUPTA
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